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Bacterial dynamics and community structure in the York River estuary

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BACTERIAL DYNAMICS AND COMMUNITY STRUCTURE IN
THE YORK RIVER ESTUARY

A Dissertation
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

by
Gary Edward Schultz, Junior
1999
APPROVAL SHEET

This dissertation is submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

Approved, April 1999

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ABSTRACT

Bacterial community dynamics were investigated over seasonal and basin scales within the York River, VA, estuary. Various parameters characterizing bacterioplankton dynamics were measured weekly at a single station (March 1996 through May 1997) and monthly at six stations (June 1996 through May 1997) spanning the entire salinity gradient (0 - ca. 20 psu over 60 km). Bacterial abundance and production were found to be high throughout the estuary. Bacterial abundance ranged from $4.4 \times 10^8$ to $1.3 \times 10^{10}$ cells-liter$^{-1}$. Incorporation of $^3$H-thymidine ranged from 10 to 863 pmol-liter$^{-1}$-hr$^{-1}$ while $^3$H-leucine incorporation rates ranged from 25 to 1963 pmol-liter$^{-1}$-hr$^{-1}$. Clear seasonal trends were apparent. The highest values of abundance and incorporation were found during the summer warm-water months. On a basin scale, bacterial properties were strongly related to changes in salinity. Bacterial properties were not related to phytoplankton biomass or production.

Although there is a great deal of variability from month to month, two opposing trends were consistently found: bacterial abundance increased from freshwater to the mouth of the river, while incorporation rates decreased from freshwater to the mouth. These patterns imply a strong landward gradient in specific growth rates. Growth rates determined by a two-dimensional box model were indeed higher upstream, but net growth rates were highest near the mouth. This explains how biomass can accumulate downstream even though production decreases.

I also used the box-model to investigate the role of physical dispersion in the distribution of bacterial cells. Dispersion rates were greater than net growth rates, indicating that dispersion controlled bacterial distribution during the study period. However, gross growth and removal rates were generally greater than dispersion rates, indicating the potential importance of biological processes to bacterial community structure within the estuary.

BIOLOG plates were used to determine if differences in bacterial community structure or metabolic capabilities occurred over time or space. Two distinct bacterial communities separated by temperature were found over the course of a year at the VIMS pier. Four distinct communities separated by temperature and salinity were found over the course of a year along the salinity gradient.

Thus, temperature, salinity, circulation and removal processes are the dominant processes controlling bacterial dynamics in the York River estuary.
PROJECT OVERVIEW

BACTERIAL DYNAMICS AND COMMUNITY STRUCTURE IN THE YORK RIVER ESTUARY
INTRODUCTION

Estuaries are defined by oceanographers as semi-enclosed coastal bodies of water, freely connected with the ocean, where seawater is measurably diluted by freshwater runoff from the mainland (Pritchard 1967). In the United States, estuaries make up 80 – 90 % of the Atlantic and Gulf coasts, and 10 – 20 % of the Pacific coast (Emery 1967). Large quantities of nutrients are brought into estuaries from the surrounding watershed resulting in high levels of primary and secondary production (Day et al. 1987). These highly productive regions provide refuge and nurseries for many finfish and shellfish (Steele 1974). Estuaries also act as filters between the land and the sea due to the intense biogeochemical processing that occurs as natural and anthropogenic materials are transported through the estuary (Head 1976, Reuter 1981, Kennedy 1986). Organic material is brought into the estuary by allochthonous inputs and is created within the estuary by autochthonous production supported by inorganic nutrients.

Bacterial abundance is high in estuaries (Ducklow and Shiah 1993), often exceeding $2 \times 10^9$ cells-liter$^{-1}$, a level seldom achieved in the open. Bacterial production in estuaries is also high, with maximum values higher than in any other marine region (Ducklow and Carlson 1992). Estuarine bacteria have been shown to utilize allochthonous inputs of vascular plant material (Coffin et al. 1989), as well as phytoplankton produced DOM (Chróst and Faust 1983, Gomes et al. 1991). Bacteria respire and remineralize organic material (Ducklow et al. 1986, Nagata and Kirchman 1991, Miller et al. 1995,
Søndergaard et al. 1995), ultimately influencing the fate of organic matter as it flows through the estuary. Bacteria may also be grazed, thus transferring their energy to at least the next trophic level within the estuary (Coffin and Sharp 1987, Sanders et al. 1989, Sherr et al. 1989). Therefore, understanding the dynamics of heterotrophic bacteria in estuaries is vital to our understanding of these ecologically important regions.

Several factors may influence bacterial dynamics within an estuary. Biological factors such as substrate supply, grazing, viral lysis, and physical factors such as temperature and circulation may influence bacterial activity and distribution. Estuaries, by nature, are dynamic regions characterized by steep gradients in salinity, nutrient concentrations, and temperature (Day et al. 1989). The complex interactions between these environmental parameters make it difficult to determine which factor or set of factors is most important in the control of bacterial dynamics. Still, several controls of bacterial parameters have been found. Temperature has been identified as an important bottom-up control on bacterial dynamics in estuaries (White et al. 1991, Hoch and Kirchman 1993, Shiah and Ducklow 1994, 1995, Tibbles 1996), while grazing by flagellates results in top-down control (Wright 1988, Billen et al. 1990).

Phytoplankton are one of the strongest influences on bacterial dynamics. In open ocean systems and in lakes, bacterial properties are often positively correlated to phytoplankton properties (Fuhrman and Azam 1980, Bird and Kalff 1984, Lancelot and Billen 1984, Cole et al. 1988, White et al. 1991, Simon et al. 1992). However, in estuaries, there is often little or no correlation between bacterial and phytoplankton properties (Painchaud and Therriault 1989, Findlay et al. 1991, Malone et al. 1991, Hoch and Kirchman 1993). As stated above, estuarine bacteria utilize phytoplankton-produced
carbon as a substrate (Chrost and Faust 1983, Gomes et al. 1991). However, several researchers showed that phytoplankton exudates in estuaries provided less than half the bacterial carbon demand (Jorgensen 1982, Lignell 1990, Malone et al. 1991). These results imply that bacterioplankton in estuaries may utilize terrestrially derived carbon. Whether or not this occurs is dependent upon the bacterial growth efficiency (BGE). If bacterial growth efficiency is low, bacterial carbon demand must be high. BGE values of 2 – 55% have been reported (Bjønnsen 1986, Billen and Fontigny 1987, Griffith et al. 1990, Kirchman et al. 1991, Coffin et al. 1993, Biddanda et al. 1994). Whether or not phytoplankton can supply all the organic material needed to meet the needs of bacterioplankton in an estuary is an important question. If phytoplankton production exceeds total respiration within an estuary, the system will be net autotrophic and organic matter will be buried within the estuary or exported out of the estuary. If system respiration exceeds primary production, the system will be net heterotrophic and the system metabolism must be supported by stored or imported organic matter (Smith et al. 1991). Understanding the trophic status of a system is important in determining the fate of DOC and POC in coastal environments (Kemp and Boynton 1992, Oviatt et al. 1993, Baines et al. 1994). The net ecosystem metabolism is also important in determining the contributions of specific systems to global carbon budgets.

Physical factors also have a large influence on bacterioplankton in estuaries. Bacteria in the water column are plankton. As such, their movement within an estuary is controlled by circulation. The average amount of time a conservative element stays within a defined volume of the estuary is defined as its residence time (Craig 1957). Freshwater input, the resulting gravitational circulation, and tidal currents control
residence time. Depending upon the ratio of the residence time to the rates of biological processes, circulation may be as important as biological processes in determining bacterioplankton abundance in an estuary. Thus, determining the extent to which physical processes affect bacterioplankton is very important in any study of estuarine bacterial dynamics.

Ketchum et al. (1952) and Ketchum (1954) first attempted to quantify dilution effects on coliform bacteria in an estuary. Other investigators have also studied the effects of physical processes on bacteria. Several studies have looked at changes in bacterial properties over tidal cycles (Erkenbrecher and Stevenson 1975, Wilson and Stevenson 1980, Wright and Coffin 1983, Kirchman et al. 1984, Shiah and Ducklow 1995). Ducklow (1982) and Koepfler et al. (1993) examined the effects of regular destratification events on bacterioplankton properties in sub-estuaries of the Chesapeake Bay. Troussellier et al. (1993) described the effects of wind perturbations on bacterial properties. However, it was not until work by Painchaud et al. (1987, 1995, 1996) that a comprehensive attempt was made to determine the effects of circulation on bacterial distribution in an estuary. Painchaud et al. (1987, 1996) used a one-dimensional box model introduced by Officer (1980) to examine how circulation affected bacterioplankton within the St. Lawrence River estuary. They found that biological processes in the St. Lawrence were as important as physical dispersion processes and that the St. Lawrence was a sink for bacteria during his study (Painchaud 1996).

Another factor that may make data that is gathered in estuaries difficult to analyze is bacterial community structure. Almost certainly the different environmental regimes and gradients present in estuaries would have different sets of active bacteria and
possibly even entirely different species. Less than 1% of marine bacteria are culturable using classical techniques (Ferguson et al. 1984). Thus, little information is available on the specific species of bacteria present in marine systems. Knowledge of the individual species present, while desirable, is not necessary to determine bacterial abundance, activity, or the factors that influence bacterial dynamics. However, the ability to identify the bacterial communities within the estuary would be very helpful in analyzing the data gathered. Molecular methods have been used to determine diversity of natural marine bacterial communities (DeLong et al. 1989, Giovannoni et al. 1990, Lee and Fuhrman 1990, 1991, Ward et al. 1990, Schmidt et al. 1991, Moyer et al. 1994, 1995). However, these methods are very time consuming and provide no information regarding which cells are active. Garland and Mills (1991) used BIOLOG plates and multivariate statistical analysis to differentiate between bacteria in a marsh creek and in an ocean inlet. BIOLOG plates use bacterial utilization of unique carbon sources to generate patterns that may be separated by multivariate statistics. BIOLOG plates are relatively inexpensive and easy to use. If BIOLOG plates are capable of differentiating communities within an estuary, they might prove to be a valuable tool to use in the study of estuarine bacterioplankton.

In 1993, Ducklow and Shiah noted that there were many studies on bacterioplankton dynamics in estuaries, but much more needed to be done. They specifically pointed out the need for seasonal studies. Until that time, only a few studies had looked at the seasonal dynamics of estuarine bacterioplankton (Larson and Hagstrom 1982, Wolter 1982, Wright et al. 1987, Newell et al. 1988). Since 1993, several other seasonal studies have examined bacterial dynamics in estuaries (Hoch and Kirchman
1993, Goosen 1997, Kirschner and Velimirov 1997). However, estuaries are quite varied in terms of temperature, freshwater flow, input of nutrients, and circulation, among other differences. Thus, there is still a need for seasonal studies of bacterioplankton in estuaries. Seasonal studies allow investigators to determine how bacterial properties are regulated over time, and allow insight into how changing environmental conditions affect bacterial communities. Due to the variety of regions, physical characteristics and differences in inputs etc., this study was designed to investigate the bacterioplankton dynamics of the York River estuary.

Study Site

The York River is a sub-estuary of the Chesapeake Bay lying in eastern Virginia between the James and Rappahannock Rivers. It begins at West Point, Virginia where it is formed by the confluence of the Pamunkey and the Mattaponi Rivers. The Pamunkey is the larger of the two rivers both in terms of freshwater discharge (USGS data) and drainage area (Cronin 1971). From West Point, the York flows southeasterly approximately 50 km to the Chesapeake Bay. Together with the Pamunkey and Mattaponi, the York drains an area of approximately 6897 km$^2$ (Cronin 1971). This study extended from the mouth of the York between the Goodwin Islands and Jenkins Neck approximately 60 km upstream to Sweethall Marsh on the Pamunkey River.

The York is broad and shallow with an average depth less than 8 m at the mouth and less than 3 m at West Point. The Pamunkey is a winding river with an average depth of ~ 5.0 m to Sweethall Marsh, with holes at each bend that are 12 to 18 m deep. The
Figure 1. Results of weekly measurements of bacterial properties at the VIMS ferry pier. Bars are bacterial properties, lines represent temperature. Bacterial abundance is shown in cells-liter$^{-1}$. Bacterial production is shown represented by TdR-incorporation (middle plot) and Leu-incorporation (bottom plot). Production is shown in pmol-liter$^{-1}$-hour$^{-1}$. Some data are missing due to hard-drive failure.
mean tidal range of the York is 0.61 m near the mouth and increases to 0.88 m at West Point before it decreases with distance upstream. Brackish water extends approximately 60 km up the Pamunkey from the mouth of the York, depending upon freshwater flow (Van Engel and Joseph 1968). The ebb excursion at the surface is longer than the flood, and the flood tide flows longest on the bottom. This indicates a weakly stratified system with net downstream transport at the surface and net upstream transport in the bottom layer (Brehmer 1970). However, destratification occurs after every spring tide due to advection of relatively fresh water into the river from the Chesapeake Bay (Haas 1977). Wind events may also cause destratification between neap-spring tidal-cycles, especially in shallower, upstream portions of the York. The water quality of the York River is influenced by an oil refinery at the mouth of the York, a paper mill at West Point, and run-off from agricultural operations along the Pamunkey and Mattaponi. Still, the York River is relatively pristine among sub-estuaries of the Chesapeake Bay (Virginia Department of Environmental Quality 1994).

Weekly sampling – VIMS pier

I began my study of the York River estuary by taking weekly samples from March 1996 to May 1997 of bacterial abundance and activity off the ferry pier on the campus of the Virginia Institute of Marine Science. Temperature ranged from 3.6° C (January 21, 1997) to 27.3° C (June 25, 1996), while salinity ranged from 11.9 °/oo (March 18, 1997) to 21.4 °/oo (January 14, 1997) (Fig. 1). Over the study period, cell numbers ranged from 1.1 x 10⁹ cells – liter⁻¹ (March 19, 1996 and February 18, 1996) to 11.0 x 10⁹ cells – liter⁻¹ (July 2, 1996). Incorporation of ³H-thymidine ranged from 7.7 – 275.2 pmol-thymidine-liter⁻¹-hour⁻¹ (January 14, 1997 and August 13, 1996,
respectively), while rates of leucine incorporation ranged between 46.3 and 2662.2 pmol-leucine-liter\(^{-1}\)-hour\(^{-1}\) (January 14, 1997 and August 6, 1996, respectively). These values of abundance and activity are high compared to open ocean measurements, but are comparable to other estuaries (Ducklow and Carlson 1992, Ducklow and Shiah 1993).

There was a great deal of variability in the measured bacterial properties from week to week (Fig. 1), but a strong seasonal signal was indicated by the positive significant linear relationship between temperature and bacterial abundance, \(^{3}\text{H}\)-thymidine incorporation and \(^{3}\text{H}\)-leucine incorporation (Table 1). No significant relationship was found between measured bacterial properties and salinity.

Other studies have shown relationships between temperature and bacterial properties (White et al. 1991, Shiah and Ducklow 1994). Jonas and Tuttle (1990) also found that bacterial abundance and activity followed the seasonal trend of temperature. Thus, it was not surprising to find a relationship between temperature and bacterial properties at the VIMS pier. However, temperature only accounts for 67% of the variance in bacterial abundance, 37% of the variance in the thymidine incorporation and 51% of the variance in the leucine incorporation (Table 1). This variability may be caused by a number of factors, including changes in the quality or quantity of substrate and grazing. Shiah and Ducklow (1993) postulated that bacterial growth in temperate estuaries was not limited by substrate. Bacterial cell abundance showed the least fluctuation among the properties measured (Fig. 1), suggesting that grazing is probably not the chief cause of the variability. Still, a scenario may be hypothesized in which...
Table 1. Results ($r^2$) of linear regressions between bacterial properties and temperature and salinity measured at the VIMS ferry pier (March 16, 1996 through May 25, 1997). Bacterial abundance and incorporation rates were natural-log transformed to achieve homoscedasticity. NS—not significant.

<table>
<thead>
<tr>
<th></th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ln abundance (cells-liter$^{-1}$)</td>
<td>0.67 p &lt; 0.01</td>
</tr>
<tr>
<td>ln TdR incorporation (pmol-liter$^{-1}$-hour$^{-1}$)</td>
<td>0.39 p &lt; 0.01</td>
</tr>
<tr>
<td>ln Leu incorporation (pmol-liter$^{-1}$-hour$^{-1}$)</td>
<td>0.57 p &lt; 0.01</td>
</tr>
<tr>
<td>Biovolume (μm$^{3}$)</td>
<td>NS</td>
</tr>
</tbody>
</table>

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grazers selectively choose more active cells. Another factor that may explain some of the variance seen in the data is physical mixing. Ducklow (1982) found that bacteria at the mouth of the York River respond to physical mixing events. This response may be a response to phytoplankton growth, which may be stimulated by redistribution of nutrients (Ducklow 1982).

One potentially important factor in the control of bacterial properties is community structure. Changes in temperature or salinity may create conditions favorable to different assemblages of bacteria. Mixing events may bring different bacteria into the study area or may change conditions at the study area to ones that favor a new set of bacteria. Any changes in community composition would not be seen by counting cell abundance or by measuring incorporation rates. Thus, changes in bacterial community composition may have also played a role in the variability seen in the measured bacterial properties.

The measurements made at the VIMS pier provided an opportunity to examine the magnitude and temporal variability in bacterial properties at one station. These results, when added to the data taken from the York River will help us determine what factors are important in the control of bacterial properties over seasonal and shorter timescales.

Objectives

My objectives were to describe patterns of bacterial dynamics along the salinity gradient as well as over a seasonal cycle in the York River estuary (Ch. 1). In addition, I wanted to determine the environmental factors that exert the largest controls on bacterial properties, and to determine if phytoplankton production in the York River was capable of meeting bacterial carbon demand (Ch. 1). Recognizing the potential importance of
physical dispersion processes in the distribution of bacterioplankton in the York River estuary, my study expands upon Painchaud’s work in the St. Lawrence River \ by modeling the York River monthly over the course of a seasonal cycle (Ch. 2). Finally, I addressed whether different communities of bacteria existed along the salinity gradient seasonally in the York River estuary (Ch. 3).

The following hypotheses guided this investigation:

H1 Bacterioplankton properties are not influenced by temperature.
H2 Bacterioplankton properties are not influenced by salinity.
H3 Bacterioplankton properties are not influenced by dissolved organic material.
H4 Bacterioplankton properties are not influenced by phytoplankton production.
H5 Physical dispersion will not control distribution of bacterioplankton cells.
H6 Bacterioplankton communities will not be differentiated by salinity.
H7 Bacterioplankton communities will not be differentiated by temperature.
CHAPTER 1

BACTERIAL DYNAMICS IN THE YORK RIVER ESTUARY
INTRODUCTION

Estuaries exist on the margins between the coastal ocean and land and mediate the export of dissolved organic carbon (DOC) and particulate organic carbon (POC) to the ocean (Winter et al. 1996). In estuarine systems, this DOC and POC may be derived from terrigenous as well as autochthonous sources. River discharge, tidal forces and other physical factors may also influence the flow of carbon and nutrients through an estuarine system. Heterotrophic bacteria are capable of consuming a large portion of autotrophic production in aquatic systems (Cole et al. 1988) as well as modifying and remineralizing organic matter. Thus, the nature and magnitude of DOC and POC export may be strongly influenced by bacterial processes. The spatial and temporal scales over which these processes operate are dependent upon the steepness of the gradients to which they are exposed and the residence time in the estuary. Depending upon freshwater input, depth and tidal energy, estuaries may have steep or shallow gradients of salinity. Many of the world's estuaries also exhibit large changes in water temperature over seasonal time scales due to their latitude and depth. Therefore, it is especially important to investigate estuarine bacterioplankton dynamics over seasonal to annual and whole-river spatial and temporal scales so that a more complete understanding of the factors controlling bacterial properties, and thus a greater understanding of the bacterial influence on organic matter in estuaries, may be obtained.
Bacterial production is the incorporation of nutrients and organic material into bacterial biomass. Production by heterotrophic bacterioplankton in estuaries is high compared to the coastal or open ocean (Ducklow and Carlson 1993, Ducklow and Shiah 1993). If bacterial carbon demand (BCD) is higher than the phytoplankton carbon produced, the bacterial metabolism of the system must be supported by stored or imported organic matter. However, if the phytoplankton production (PP) is larger than bacterial carbon demand, then organic matter is either buried or exported. Determining the ratio between BCD and PP in an ecosystem is important in assessing the local net export or deposition of POC (Kemp and Boynton 1992, Oviatt et al. 1993, Baines et al. 1994). On a global scale, finding the net ecosystem production to respiration (P:R) ratio in specific systems is useful in understanding large-scale carbon budgets (e.g. Smith and Hollibaugh 1993).

In most studies of non-estuarine aquatic ecosystems, bacterial production was positively correlated with primary phytoplankton production (Fuhrman and Azam 1980, Bird and Kalff 1984, Lancelot and Billen 1984, Bjornsen et al. 1988, Cole et al. 1988, Currie 1990, Simon et al. 1990, White et al. 1991). This implies that primary production ultimately provides most of the carbon needed for bacterial production. This is probably true of oceanic systems and lakes with few other local sources of carbon for bacteria to use. However, in many estuarine systems, the relationship between bacterial and primary production is often weak or non-existent (Albright 1983, Ducklow and Kirchman 1983, Wright and Coffin 1983, 1984, Coffin and Sharp 1987, Painchaud and Therriault 1989, Findlay et al. 1991, Malone et al. 1991, Ducklow and Shiah 1993; but see Goosen et al. 1997). This may be due to the existence of important non-phytoplankton created
substrates in most estuaries. Coffin et al. (1989) used isotopic composition to show that estuarine bacteria do, in fact, use substrates from a variety of primary producers other than phytoplankton including upland C-3 plants and Spartina.

The relationship between phytoplankton production and bacterial production is further complicated when temporal effects are included. Billen (1990) and Ducklow et al. (1993) have observed that bacterial biomass and production may lag a few weeks behind increases in phytoplankton production and biomass. Of the few studies of estuarine systems lasting an entire year or longer, none reported high covariation between bacterial and phytoplankton properties (Wright et al. 1987, Ducklow and Shiah 1993, Hoch and Kirchman, 1993). Limits on biomass and abundance are thought to be set by top-down controls (Wright 1988, Billen et al. 1990), while limits on bacterial growth rates are thought to be set by bottom-up factors such as substrate supply and temperature (Hoch and Kirchman 1993, Shiah and Ducklow 1994, 1995, White et al. 1991). In an estuary, these factors may vary seasonally as they are influenced by biological as well as physical processes such as freshwater flow, light intensity, storm frequency, circulation effects and other physical processes (Day et al. 1989). Thus, it is important to study bacterial dynamics over varying time scales to understand the effects of these factors on bacterial production.

The tributaries of the Chesapeake Bay play an important role in the water quality of the Bay. Despite that, there are few reports of bacterial observations on the major tributaries of the Chesapeake Bay. Ducklow (1982) studied the effect of the spring-neap tidal cycle on bacterial abundance and activity at the mouth of the York and Koepfler et al. (1993) examined spring-neap effects on microbial populations in the James River.
There have been no other studies of bacterial dynamics in the major tributaries of the Chesapeake Bay. In this study, I set out to begin to correct this paucity of data by describing the basic spatial and temporal patterns of heterotrophic bacterioplankton abundance and activity in the York River estuary. In doing so, I sought to determine those environmental factors that were most strongly related to, and thus potentially control, bacterial abundance and production. Here I present data on monthly, seasonal and annual mean patterns of bacterial abundance and production in the York River, VA estuary.

**MATERIALS AND METHODS**

**Study site**

The York River is a sub-estuary of the Chesapeake Bay between the James and Rappahannock Rivers with a drainage area of 6897 km$^2$ (Cronin, 1971). The York flows in a southeasterly direction approximately 50 km from the confluence of the Pamunkey and Mattaponi rivers at West Point, Virginia to the mouth near Yorktown, Virginia, where it empties into the Chesapeake Bay (Fig. 1). The Pamunkey is larger than the Mattaponi with a drainage area of approximately 3748 km$^2$. Salt and freshwater marshes border the Pamunkey throughout the study area. The entire sampling area is tidally influenced with a mean tidal range of 0.61 m at the mouth of the York, increasing to 0.88 m at West Point before decreasing again (Barden, 1930). Brackish water extends approximately 60 kilometers upstream. There is an oil refinery at the mouth of the York, a paper mill at West Point, and runoff from agricultural operations that influence the
Figure 1. Map of site locations (stations 1 – 6). York River estuary, VA.
water quality in the York and Pamunkey Rivers. However, the York River is still one of the most pristine sub-estuarine systems in the entire Chesapeake Bay system (Virginia Dept. of Environmental Quality 1994).

Sample collection

Samples were collected monthly at six stations along the salinity gradient of the York River (Fig. 1) aboard the RV 'Kingfisher' from June 1996 through May 1997. Samples were then collected every other month from July 1997 to May 1998. Stations were approximately 9 km apart with Station 1 located at the mouth of the York and Station 6 located in freshwater (0°/oo) in the Pamunkey River approximately 60 kilometers from the mouth. Samples were collected at each station using a clean (acid washed) 2.5 liter Niskin bottle one meter below the surface and one meter above the bottom, for determination of bacterial cell abundance, bacterial activity, chlorophyll a, temperature, salinity, dissolved organic carbon (DOC), dissolved organic nitrogen (DON), dissolved oxygen (DO), total dissolved nitrogen (TDN), nitrate (NO₃⁻), nitrite (NO₂⁻) and ammonium (NH₄⁺).

Bacterial cell abundance and biovolume measurements

Samples for bacterial abundance and biovolume were preserved with 0.2 μm filtered 25% glutaraldehyde (Sigma) diluted to a final concentration of 2%. Preserved samples were then stored at 4° C until slide preparation. Slides were prepared within seven days of collection. Two (2) to six (6) mls of sample were filtered onto 0.2 μm black polycarbonate filters (Poretics). A mixed-ester backing filter (MSI) was used to ensure even distribution of cells on the slide. As the samples were being filtered, 200 μl
of acridine orange (Sigma) solution (final concentration 0.005%) were added to stain the cells for viewing (Hobbie et al., 1977). Once the sample had completely filtered through, but before the filter could become dry, the black polycarbonate filter was removed from the filter housing and mounted on a glass slide in Resolve™ immersion oil and frozen until examination.

All slides were enumerated by epifluorescence microscopy. A Zeiss Axiophot microscope at 1613 x with a blue BP 450 – 490 excitation filter and an LP-520 barrier filter was used. To estimate biovolume, images were taken with a Dage-MTI Nuvicon video camera connected to the Axiophot microscope through a Dage Gen-II image intensifier. Images were processed and analyzed using the PC 486-based Zeiss Vidas Videoplan Image Analysis system. Fluorescent spheres of various sizes (Polysciences Corp.) were used for calibration. Cell volumes were estimated using the algorithm of Baldwin and Bankston (1988).

**Bacterial production – 3H-thymidine and 3H-leucine incorporation**

Bacterial production was estimated from [\(^3\)H-methyl]-thymidine (\(^3\)H-TdR) and [\(^4,5\)-\(^3\)H]-leucine (\(^3\)H-Leu) incorporation. Thymidine is incorporated directly into DNA, and is thus a measure of cell division, while leucine is incorporated into protein and is a measure of protein synthesis. \(^3\)H-TdR (Dupont, specific activity ≈ 80 Ci/mmol) was added to forty-eight 2.0 ml centrifuge tubes and \(^3\)H-Leu (Dupont, specific activity ≈ 180 Ci/mmol) was added to another forty-eight 2.0 ml centrifuge tubes prior to each cruise. The tubes were kept on ice until needed. The concentrations of \(^3\)H-TdR and \(^3\)H-Leu were such that 10 µl of isotope solution with 1.7 ml of sample gave a final concentration of 25 nM thymidine and 40 nM leucine. At each station, four tubes with \(^3\)H-TdR and
four tubes with $^3$H-Leu were used. One of the four tubes (of each treatment) was made a blank by the addition of 100 μl of ice-cold 100% trichloroacetic acid (TCA; Fisher) prior to addition of sample. All tubes were pre-filled with isotope solution and taken out on each cruise on ice.

Once water was collected, 1.7 ml of sample were added to each of four tubes with $^3$H-TdR and to each of four tubes containing $^3$H-Leu (three replicates and a blank), to initiate incubation assays. Samples were turned over to mix and then placed into water in an insulated container at near in situ temperature. Samples were allowed to incubate approximately one hour, at which time the incubations were stopped by adding 100 μl of 100% TCA to the centrifuge tubes and gently mixing by inversion. Samples were then placed on ice until return to the lab.

The maximum time between sampling the first station and return to the lab was less than 8 hours. Samples were processed immediately upon returning to the lab. Processing consisted of spinning the samples in a high speed refrigerated microcentrifuge (Eppendorf, model 5402) at 14000 rpm for seven minutes at 2°C to create a pellet containing bacterial cell walls and molecules too large to pass through the membranes compromised by the TCA addition (DNA, RNA and proteins). Then, the supernatant was aspirated and 1.8 ml of 5% ice cold TCA were added. The samples were spun again for seven minutes and the supernatant was aspirated. Finally, 1.8 ml of 80% ethanol (Fisher) were added. The samples were spun a final time and the supernatant was aspirated. One ml of Ultima Gold scintillation cocktail (Packard) was added and the radioactivity of the samples was counted in a liquid scintillation counter (Wallac, model 1409).
To determine the sampling precision of the data collected, triplicate samples were collected in March 1998 at stations 2 and 5 as well as on several other occasions off the VIMS pier. For bacterial cell abundance, cell biovolume, thymidine incorporation rates (TdR-incorporation) and leucine incorporation rates (Leu-incorporation), the coefficient of variation between the three replicate samples in most cases was lower than 10% and in all cases lower than 15%.

Conversion factors

To estimate bacterial production values from incorporation of thymidine and leucine, conversion factors must be used (Ducklow and Carlson 1992). To find bacterial cell production from TdR-incorporation, I used the conversion factor for an estuary of 1.1 x 10^{18} cells/mole of thymidine incorporated (Riemann et al. 1987). To obtain production values in carbon units from TdR-incorporation, it is also necessary to use a carbon conversion factor to convert from cell volume or cell number to units of carbon. There are a wide variety of published values from which to choose (Ducklow and Carlson 1992). To ensure that differences in cell size within the estuary were incorporated into the estimated production values, a volumetric conversion factor was chosen. I chose to use the carbon conversion factor of 120 fg C \mu m^3 published by Watson et al. (1977) because it is a measured value and for consistency (Carlson and Ducklow 1996, Carlson et al. 1996). Thus, bacterial production values were calculated from TdR incorporation and average cell biovolume for each sample.

Theoretically, the amount of leucine in protein and the ratio of cellular carbon to protein should be constant for cells in balanced growth (Simon and Azam, 1989). Thus, using the best estimates for these numbers, a conversion factor of 3.1 kgC/mol leucine is
obtained. This factor was used to convert from leucine incorporation to bacterial production.

**Primary production**

I did not measure phytoplankton primary production, but data are available from the EPA's Chesapeake Bay Monitoring Program (http://www.chesapeakebay.net). Phytoplankton production numbers presented here are based on $^{14}$C fixation rates obtained from replicate surface layer samples collected monthly from June 1996 through November 1997. Rates were measured according to Strickland and Parsons (1972) from samples collected at three sites in the York River. These sites were designated by the EPA as freshwater zone, transition zone and mouth. These three stations are located approximately at my stations 6, 5 and 1 respectively.

To obtain estimates of primary production for stations 4, 3, and 2, I interpolated from data measured at stations 5 and 1 by assuming a linear change in phytoplankton production from station 5 to station 1. Using mean light intensity data (Sin, personal communication), an average euphotic zone depth was determined for each station (3.1 m at station 1, 1.3 m at station 5 and 1.4 m at station 6). Using the measured and interpolated phytoplankton production values, the estimated euphotic zone depth, and the published surface areas and volumes associated with each station within the York (Cronin, 1971), values for integrated primary production for each region of the estuary were determined.
Chlorophyll $a$

Samples for chlorophyll $a$ were taken at each station (surface and bottom). Approximately 50 – 100 ml of sample were collected in a 2.5 l Niskin bottle and transferred to polycarbonate bottles. The bottles were stored on ice until returned to the lab. Chlorophyll $a$ was determined by DMSO/acetone extraction according to Burnison (1980). Triplicate aliquots of eight ml were filtered through GF/F (Whatman) filters. The filters were placed into darkened test tubes filled with 8 ml of a DMSO/acetone/water solution (45:45:10). Test tubes were kept sealed and in the dark for at least 48 hours at room temperature. Samples were poured from the test tubes into clean glass cuvettes and the fluorescence read on a fluorometer (Turner Designs, model 10-AU). A test tube filled with the DMSO/acetone/H$_2$O solution but with no filter was used as a blank to zero the fluorometer prior to use. The fluorometer was calibrated before sampling began in 1996 and again one year later in 1997.

Environmental parameters

The Analytical Lab at the Virginia Institute of Marine Science analyzed dissolved organic matter and inorganic nutrients, which were collected and measured as follows:

Approximately 250 ml of sample were collected to measure dissolved oxygen (DO) concentration in a glass bottle. Care was taken to ensure that no air bubbles adhered to the bottle walls. Immediately after obtaining the sample, > 1 ml of manganous chloride (600 g MnCl$_2$-4H$_2$O in 1000 ml H$_2$O) followed by > 1 ml of sodium iodide-sodium hydroxide solution (600 g NaI with 320 g NaOH with enough water added to make a 1000 ml solution) were added to each sample. The top was carefully replaced ensuring that no air was left in the bottle. The bottle was then mixed by inversion and
stored at ambient temperature until return to the lab. Samples were analyzed using Winkler titration (Carpenter, 1965).

Samples for salinity were also collected in a 250 ml glass bottle and stored at room temperature. Approximately 500 ml of sample were also collected in a baked glass bottle for dissolved organic carbon analysis (DOC). DOC was determined by high temperature catalytic oxidation techniques (Williams et al., 1993) using a Shimadzu TOC-5000.

Another 500 ml was collected in a polycarbonate bottle for dissolved organic nitrogen (DON) analysis. DON was measured by catalytic oxidation (Williams et al., 1993) followed by determination of $\text{NO}_2^-$ and $\text{NO}_3^-$. Samples for determination of ammonium ($\text{NH}_4^+$), total dissolved nitrogen (TDN) and $\text{NO}_2^-$ and $\text{NO}_3^-$ concentrations were also taken from the polycarbonate bottle containing the DON sample. $\text{NH}_4^+$ was determined by the phenolhypochlorite method (Parsons et al., 1984). TDN was determined by persulfate digestion (Parsons et al., 1984). Finally, $\text{NO}_2^-$ and $\text{NO}_3^-$ concentrations were determined by Cd-Cu reduction (Parsons et al., 1984). All samples other than those for oxygen and salinity were stored on ice until return to the lab (no longer than 8 hours).
RESULTS

Physical and chemical properties of the York River estuary

In most cases, bottom water properties mimicked surface water properties. All general trends reported below are for both surface and bottom waters unless otherwise noted.

For the purposes of this study, the York River was sampled at 6 stations along the salinity gradient (Fig. 1). Salinity varied at each station over the course of the study (Fig. 2). A distinct peak in salinity at each station in September 1997 was due to an extended drought through the late summer of 1997. There was also a sharp decline in salinity in the spring of 1998. For any given time period, water temperature of the York varied less than 2°C from station to station (Fig. 2). Seasonally, temperature ranged from ~ 5°C in the winter months to greater than 25°C in the summer.

DOC concentrations ranged from 2.8 to 8.9 mgC-liter$^{-1}$ (January 1998, station 1 and September 1997, station 1; respectively) and were generally higher in the summer and fall than in the winter and spring (Fig. 2). DOC tended to increase from station 1 to station 6. DON concentrations ranged from 0.15 to 0.52 mgN-liter$^{-1}$ (January 1998, station 1 and September 1997, station 1; respectively). DON concentrations at all stations increased sharply between July and August 1996 (Fig. 2), then decreased to a low in February 1997, before peaking in September 1997. Compared to DOC, there was little evident difference spatial variation in DON concentration in most months. DON and DIN concentrations showed opposing trends over the two-year period (data not shown). Patterns of TDN, NO$_2^-$ and NO$_3^-$, and NH$_4^+$ concentrations were typical for the York River (data not shown; Sin et al. 1999).
Figure 2. Surface values of temperature, salinity and chlorophyll a over the course of the sampling period (June 1996 – May 1998). Numbers in the upper right of each graph indicate station number. Ranges are held constant for all properties at all stations to show station to station differences.
Figure 2. (cont.) Surface measurements of DOC and DON over the course of the sampling period (June 1996 – May 1998). Numbers in the upper right of each graph indicate station number. Ranges are held constant for all properties at all stations to show station to station differences.
Chlorophyll \(\alpha\)

Chlorophyll \(\alpha\) concentrations in the York River ranged from 2.5 to 178.7 \(\mu\text{gC-liter}^{-1}\) (December 1996, station 6 and September 1997, station 4; respectively) (Fig. 2). A phytoplankton bloom occurred in the upper river (stations 4, 5 and 6) in May through October 1997. Another occurred at stations 3 and 4 in February and March 1997. Sporadic smaller scale blooms occurred at other times and locations (Fig. 2).

Bacterial properties of the York River: temporal patterns

Bacterial cell abundance exhibited a seasonal pattern at all stations (Fig. 3). Cell abundance was greatest during the summer months and lowest during the winter months. The greatest measured cell abundances occurred in the first few months of the study (June and July 1996). Bacterial production (as measured by TdR-incorporation) showed the same general pattern as bacterial abundance, with the largest TdR-incorporation rates occurring in the spring of 1998 (Fig. 3). Leucine incorporation rates were also highest in the summer and lowest in the winter, with the highest Leu-incorporation rates found in July of 1997 (Fig. 3). TdR-incorporation and Leu-incorporation were divided by the number of bacterial cells to obtain a measure analogous to growth rate. TdR/cell was highest in the fall 1996 and spring 1998 and increased in magnitude from station 1 to station 6 (Fig. 3). Leu/cell values were variable from month to month. Leu/cell also tended to increase with distance upstream (see below).
Figure 3. Bacterial abundance, thymidine incorporation rate, and leucine incorporation rate at each station over the sampling period (June 1996 – May 1998).

Station numbers are in the upper right of each chart. Values represent samples collected one meter below the surface. Bottom values typically follow surface values. Ranges are held constant for all properties at all stations to show station to station differences.

Thymidine incorporation rate for May 1998 sample is off the chart (798 pmol-l\(^{-1}\)-hr\(^{-1}\)).
Cells-litter⁻¹ (x 10⁶)

St 1

St 2

St 3

St 4

St 5

St 6

TDR (pmol-l⁻¹ hr⁻¹)

Leu (pmol-l⁻¹ hr⁻¹)
Figure 3 (cont.). Thymidine and leucine incorporation rate per cell ($10^{-20}$ mol-cell$^{-1}$-hr$^{-1}$) at each station over the sampling period (June 1996 – May 1998). Station numbers are in the upper right of each chart. Values represent samples collected one meter below the surface. Ranges are held constant for all properties at all stations to show station to station differences.
Bacterial properties of the York River: spatial patterns

During every sampling period, bacterial cell abundance at the surface generally decreased with distance upstream from the mouth of the river (Fig. 4). TdR-incorporation (i.e. production), however, displayed the opposite trend. Although the maximum was occasionally at station 4 or 5, every month sampled showed a general increase in surface layer TdR-incorporation with distance upstream (Fig. 4). Although not as pronounced, this pattern was also seen in the bottom layer (data not shown). Leucine-incorporation was quite variable from station to station and exhibited no discernable upstream/downstream pattern. TdR/cell also tended to increase with distance upstream from the mouth, indicating an increase in growth rates, while leucine/cell showed no pattern with distance upstream.

Relationships between bacterial properties and chemical and physical factors

To begin the process of determining the chemical and physical factors that may control bacterial abundance and activity within the York River estuary, correlations were performed among all measured parameters using both surface and bottom data (Table 1). No strong correlations (i.e. $r \geq 0.7$) were found between bacterial properties and the environmental data using all the data together. Bacterial abundance was significantly correlated with the environmental parameters NO$_2$ and NO$_3$, DO, temperature and salinity. Only the correlation with temperature had an $r$ value $\geq 0.5$. TdR was correlated with DOC, DON, DO, temperature and salinity, but none of the correlations had an $r$ value $\geq 0.5$. Leu was also correlated with DOC and DON as well as NO$_2$ and NO$_3$, DO and temperature. Leucine incorporation rate was not significantly correlated with salinity, but showed a fairly strong correlation with temperature ($r = 0.79$, $p < 0.01$).
Figure 4. Monthly measurements of bacterial properties (June 1996 to November 1996). Station number is x-axis. Units are: abundance (x $10^9$ cells-l$^{-1}$), TdR incorporation (pmol-l$^{-1}$-hr$^{-1}$), Leu incorporation (pmol-l$^{-1}$-hr$^{-1}$), TdR/cell (x $10^{-20}$ mol-cell$^{-1}$-hr$^{-1}$), Leu/cell (x $10^{-20}$ mol-cell$^{-1}$-hr$^{-1}$). Values represent samples collected one meter below the surface. Ranges are held constant for all properties at all stations to show station to station differences.
Figure 4 (cont.). Monthly measurements of bacterial properties (December 1996 to May 1997). Station number is x-axis. Units are: abundance ($\times 10^9$ cells-l$^{-1}$), TdR incorporation (pmol-l$^{-1}$-hr$^{-1}$), Leu incorporation (pmol-l$^{-1}$-hr$^{-1}$), TdR/cell ($\times 10^{-20}$ mol-cell$^{-1}$-hr$^{-1}$), Leu/cell ($\times 10^{-20}$ mol-cell$^{-1}$-hr$^{-1}$). Values represent samples collected one meter below the surface. Ranges are held constant for all properties at all stations to show station to station differences.
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Figure 5. Scatter plots to show relationships between bacterial properties (abundance, TdR incorporation, and Leu incorporation) against temperature, salinity and chlorophyll $a$. 
Table 1. Results (r values) for correlations calculated between bacterial properties and environmental parameters on all data (surface and bottom) collected in the York River estuary from June 1996 through May 1998. Bold type indicates that the correlation was significant (p < 0.01).

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<th>Leu</th>
<th>TdR/Cell</th>
<th>Leu/Cell</th>
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<th>DON</th>
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</table>
Figure 5 shows scatter plots of raw data for bacterial properties against temperature, salinity and chlorophyll a segregated into surface data, bottom data and all data. To determine the factors that were together best correlated with the bacterial properties, best subsets regressions were run on the entire unsorted data set using Minitab (Minitab, Inc., v12.1). Best subsets regressions find the strongest linear regression between a dependent variable and a set of independent variables. Then two-factor linear regressions were run to find the two variables with the strongest relationship with the dependent variable. This process was repeated as many times as there were independent variables. These best subsets regressions indicated that salinity and/or temperature were the two factors most related with the bacterial properties (data not shown). Shiah and Ducklow (1994) showed that bacterial abundance and activity might be controlled by temperature. Painchaud et al. (1995) showed that increases in salinity might reduce bacterial growth rate by 50%. Because of this possible causal relationship, linear regressions rather than correlations were used to test for relationships (Table 2) between these two factors and the bacterial properties. Due to the weakly stratified nature of the York, the raw data were separated into surface and bottom components before linear regressions were performed.

The results of these regressions again demonstrated few strong relationships ($r \geq 0.7$) in the raw data (Table 2). Temperature and salinity together accounted for 50% of the variance in abundance at the surface. Temperature accounted for 63% of the variance in Leu in both surface and bottom waters. There were no other relationships with an $r^2 > 0.5$. 

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Figure 6. Relationships between the means of data from all six stations for each month sampled (surface and bottom data, June 1996 – May 1997 only) and temperature, salinity and chlorophyll a. Bacterial properties were natural log transformed before plotting against temperature. Abundance values plotted against salinity and chlorophyll a are x 10^9 cells-liter^-1. Incorporation rates plotted against salinity and chlorophyll a are in pmol-liter^-1-hour^-1. Error bars indicate standard error.
Table 2. Results of linear regressions \((r^2)\) run between bacterial properties and temperature and salinity (significance level is \(p < 0.01\)). Data were analyzed using all stations and months together, but separated by depth.

<table>
<thead>
<tr>
<th></th>
<th>Temp Surface</th>
<th>Salinity Surface</th>
<th>Temp &amp; Salinity Surface</th>
<th>Temp Bottom</th>
<th>Salinity Bottom</th>
<th>Temp &amp; Salinity Bottom</th>
<th>Temp Surface &amp; Bottom</th>
<th>Salinity Surface &amp; Bottom</th>
<th>Temp &amp; Salinity Both</th>
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<tr>
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<td>.18</td>
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<tr>
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<td>NS</td>
<td>.63</td>
<td>NS</td>
<td>.62</td>
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<td>Leu/cell</td>
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<td>NS</td>
<td>.41</td>
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<td>.13</td>
<td>.41</td>
<td>.30</td>
<td>.09</td>
<td>.41</td>
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</tbody>
</table>
Table 3. Temporal patterns. Results of linear regressions ($r^2$) run between the means of bacterial properties at all stations for each month of the sampling period. Bacterial abundance and incorporation rates were natural-log transformed to achieve homoscedasticity. Significance level is $p < 0.01$. NS – not significant.

<table>
<thead>
<tr>
<th></th>
<th>Temperature surface</th>
<th>Temperature bottom</th>
<th>Temperature surface &amp; bottom</th>
</tr>
</thead>
<tbody>
<tr>
<td>ln Abund</td>
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<td>.57</td>
</tr>
<tr>
<td>ln TdR</td>
<td>.64</td>
<td>.51</td>
<td>.57</td>
</tr>
<tr>
<td>ln Leu</td>
<td>.85</td>
<td>.80</td>
<td>.83</td>
</tr>
<tr>
<td>ln TdR/cell</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ln Leu/cell</td>
<td>.52</td>
<td>.48</td>
<td>.54</td>
</tr>
</tbody>
</table>
Temporal relationships

To examine relationships over temporal scales, linear regressions were run between the station means of the data grouped by month (Table 3). This transformation of the data allowed me to reduce spatial salinity gradients and examine seasonal patterns in the data (Fig. 6). Correlations were first run between all parameters (data not shown). The only significant relationships between environmental factors and bacterial properties other than temperature were between Leu and Leu/cell and DOC and DON. These correlations were not strong ($r < 0.6$) and are probably due to autocorrelation with salinity. Therefore, linear regressions between bacterial properties and temperature alone were run (Table 3).

Mean bacterial abundance over the entire estuary was positively and significantly related to temperature in the surface waters, bottom waters and in both. Temperature explained 57% of the variance in abundance over time (surface and bottom) (Fig. 6). Leucine was also positively and significantly related to seasonal increases in temperature, with temperature accounting for more than 83% of the variance. TdR was also significantly related to temperature, with an $r^2 = 57\%$. TdR/cell was not significantly related to temperature, but Leu/cell was significantly related to changes in temperature (Table 3).

Spatial relationships

Relationships along the salinity gradient were examined by performing linear regressions between the sampling period mean values grouped by station. Linear regressions between bacterial properties and salinity were run (Table 4). Examining
Figure 7. Relationships between the means of data over the entire two-year sampling period at each station (surface and bottom data) and temperature, salinity and chlorophyll $a$. Numbers indicate station number. Error bars indicate standard error.
these results allowed me to determine if patterns along the length of the estuary existed (Fig. 7) by removing much of the influence of seasonal changes in temperature.

Bacterial abundance was significantly and positively related to salinity along the estuary (Fig. 7). Salinity explained 97% of the variance in abundance at the surface, 87% in the bottom layer and 85% using both sets of data. TdR was also very strongly related to salinity. TdR was significantly and negatively related to salinity in surface, bottom, and surface plus bottom waters ($r^2 = 0.999$, $r^2 = 0.99$ and $r^2 = 0.99$ respectively, $p < 0.001$ for all). The rate of bacterial incorporation of leucine, however, was not significantly related to salinity. TdR/cell and Leu/cell were both significantly and negatively related to salinity.

**Seasonal trends in TdR/cell and Leu/cell**

The year was divided by temperature and season into four periods. Winter months were defined as those months when temperature was less than 10° C (December 1996, January 1996 and 1997, February 1997), spring and fall were defined as those months with temperature from 10 to 25° C (spring; March 1997 and 1998, April 1997, May 1997 and 1998: fall; September 1996 and 1997, October 1996, November 1996 and 1997), and summer months were those in which the water temperature was > 25° C (June 1996, July 1996 and 1997, August 1996). Seasonal averages for TdR/cell and Leu/cell were calculated for each station (Table 5). TdR/cell were lowest in winter and highest in the fall and spring at all stations except station 4 (Fig. 8). Leu/cell generally showed the same pattern as TdR/cell rates (Table 5).
Figure 8.  Seasonal bacterial activity measured as TdR incorporated-cell$^{-1}$-hr$^{-1}$. Fall values are the mean at each station for the months of September, October, and November (temperature between 10° C and 25° C); winter values are the mean at each station for the months of December, January, and February (temperature < 10° C); spring values are the mean at each station for the months of March, April, and May (temperature between 10° C and 25° C); and summer values are the mean at each station for the months of June, July, and August (temperature > 25°C). Error bars are standard error.
Figure 9. Integrated values for bacterial production (BP) and phytoplankton production (PP) at stations 6, 5 and 1. Bacterial production calculated from TdR-incorporation and Leu-incorporation. Data are shown from the beginning of the sampling period (June 1996) through all months in which EPA phytoplankton production data were available as of January 1998.
Table 4. Spatial patterns. Results of linear regressions ($r^2$) run between the means of bacterial properties over the sampling period at each station against salinity. Significance level is $p < 0.01$. NS – not significant.

<table>
<thead>
<tr>
<th></th>
<th>Salinity surface</th>
<th>Salinity bottom</th>
<th>Salinity surface &amp; bottom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abund</td>
<td>.97</td>
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<td>.85</td>
</tr>
<tr>
<td>TdR</td>
<td>.999</td>
<td>.99</td>
<td>.99</td>
</tr>
<tr>
<td>Leu</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TdR/cell</td>
<td>.93</td>
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<td>.88</td>
</tr>
<tr>
<td>Leu/cell</td>
<td>.99</td>
<td>.84</td>
<td>.79</td>
</tr>
</tbody>
</table>
**Bacterial production versus phytoplankton production**

To compare bacterial and primary phytoplankton production I used data from the EPA Chesapeake Bay Monitoring data set (http://www.chesapeakebay.net) over the sampling period. Neither bacterial abundance nor bacterial production (based on TdR incorporation rates) was significantly correlated to primary production (data not shown).

At the freshwater station (station 6), phytoplankton production was relatively low throughout the sampling period (Fig. 9). There was a small maximum in July 1996 and another larger maximum in July 1997. At the transition zone, station 5 primary production was low through the year until a bloom in April 1997 (Fig 9) and then stayed relatively high throughout the rest of the summer. At the mouth of the York River, station 1, phytoplankton production rates were higher than at either stations 5 or 6 and there was a peak in primary production from January 1997 through April 1997 (Fig. 9).

Bacterial production (calculated from TdR-incorporation) at station 6 peaked in October 1996 with another small peak in December 1996. Over most of the year, bacterial production was greater than primary production at station 6 (Fig. 9). At station 5, there were three peaks in TdR-based bacterial production. One in July 1996, another in January 1997 and the third in July 1997. Bacterial production at station 5 was generally lower than primary production through the sampling period. At station 1, integrated bacterial production exhibited small peaks in August 1996, April 1997 and again in July of 1997. Again, integrated bacterial production at station 1 was generally much less than primary production (Fig. 9).
DISCUSSION

Control of bacterial properties in the York River estuary

The York River demonstrates quite clearly the problems inherent in determining the factors controlling bacterial abundance, activity and growth rate in an estuary. So many factors are changing simultaneously, that determining the one or two parameters that exert the greatest control over bacterial dynamics is not straightforward. However, examination of the data does show that bacteria in the estuary have their lowest values of abundance, activity and growth rate during the cold-water winter months. In general, abundance and activity do not increase until the temperature rises in the early spring. Bacterial measurements did not correlate to either phytoplankton biomass (chlorophyll a) or to primary production. This is not unusual for estuaries, especially in this region (Wright and Coffin 1984, Findlay et al. 1991, Ducklow and Shiah 1993), indicating that estuarine bacterial dynamics cannot be evaluated in the same fashion as open ocean bacterial dynamics. Bacterial abundance and activity may actually be influenced by phytoplankton dynamics, but other processes may blur or shift this relationship. For example, the resuspension of sediments in shallow areas due to wind waves can be significant (Anderson 1972). Resuspension of detrital phytoplankton material from the benthos may give misleading measurements of the current conditions.

Of all the factors measured in this study, changes in bacterial abundance were most closely related to changes in salinity and temperature. The concentrations of organic substrates (DOC, DON) were not significantly related to bacterial properties. Bacterial dynamics in the York River estuary may be dependent upon the labile fraction of these materials, but changes in the concentrations of the labile portions may be masked...
by the bulk properties (total DOC, DON) measured. Another possibility is that the concentrations of these substrates are high enough that they are always in excess and bacteria are limited by other factors, such as inorganic nutrients or temperature.

It is well established that temperature is important in the control of estuarine bacterial dynamics (Ducklow and Shiah 1993, Hoch and Kirchman 1993, Shiah and Ducklow 1994). The relationships between bacterial properties and temperature are also strong in the York River estuary over the seasonal time scales (Table 3). Abundance, Leu, Leu/cell and TdR are strongly related to temperature, but TdR/cell is not significantly related to temperature (Table 3). TdR/cell was highest in the spring and fall months (Fig 8) when the water temperature is warm, but not the warmest it will become. The difference in the spring/fall months and in summer months may be in labile DOM. In the spring, rainfall usually increases and river discharge is greater, bringing more run-off and thus more nutrients into the system. In the fall, river discharge is low, but upland plants are losing large amounts of organic material that is washed into the rivers and streams to be brought to the estuary. Coffin et al. (1989) showed that bacteria could make use of upland plant matter. Although it is not clear that there are greater amounts of DOC and DON in the river in the spring and fall (Fig. 2), the labile fraction of the DOC and DON measured may be greater during these months. Such inputs of allochthonous material have been shown to disrupt the relationship between bacterial and phytoplankton properties (Kirchman et al. 1989, Painchaud and Therriault 1989, Findlay et al. 1991). This may help explain the lack of correlation between phytoplankton and the bacterial properties.
Although temperature seems to be the factor with the greatest control over bacterial properties on the seasonal time scale, it does not explain all of the variance in the data. Other factors must play roles in the dynamics of bacteria in the estuary. As discussed above, utilizable substrate supply may also play an important role in seasonal control of bacterial dynamics, but I was unable to resolve this question from the data in hand. Also, over spatial scales, the small differences in temperature within the estuary at any given moment cannot govern bacterial properties.

Inverse patterns of abundance and activity

The York River is similar to other estuaries that have been studied in that bacterial abundance and activity are quite high throughout the estuary. However, upon perusal of the data, a surprising pattern becomes apparent not reported in other estuaries. Bacterial abundance in estuaries sometimes decreases downstream (Palumbo and Ferguson 1978, Painchaud and Therriault 1989), sometimes increases downstream (Christian et al. 1984, Coffin and Sharp 1987) and sometimes exhibits mid-estuarine peaks (Prieur et al. 1984, Wright and Coffin, 1983). But in all cases reported, bacterial activity tends to correlate positively with bacterial abundance. This is not the case in the York River estuary. In every month sampled, (eighteen total) the York demonstrated opposite trends between bacterial abundance and activity along the salinity gradient (Fig. 10). This pattern implies a strong landward gradient in growth rates. In fact, TdR/cell does increase significantly with decreasing salinity (Table 3 and Fig. 11). This pattern is not only true of the annual means, but in the surface waters, this general pattern between bacterial abundance and TdR occurred at every sampling period (Fig. 4). Leucine incorporation rates were not significantly related to salinity and did not show this pattern (Table 3 and
Figure 10. Inverse pattern of bacterial abundance and production (TdR-incorporation). Data plotted are the mean values of bacterial abundance and production over the entire two-year sampling period at each station. Error bars are standard error.
Figure 11. Specific growth rate (TdR-incorporation-cell\(^{-1}\)-hr\(^{-1}\)). Data shown are means over the sampling period at each station. Error bars are standard error.
Figure 12. Mean values of carbon per newly created cell (C/NCC) over the entire two-year sampling period at each station (x $10^{-15}$ gC-cell$^{-1}$). There is a significant difference between the C/NCC at station 1 and station 6 in both the surface and bottom waters. (Student’s t-test, p < 0.001).
Fig. 4). Possible reasons for this pattern will be discussed below after examining the possible consequences.

The amount of carbon per newly created cell (NCC) is obtained by dividing the leucine production (in g C·t⁻¹·hr⁻¹) by TdR after multiplying TdR by the thymidine conversion factor (in this case 1.1 x 10¹⁸ cells/mole thymidine) (i.e. gC·liter⁻¹·hour⁻¹/cells produced-liter⁻¹·hour⁻¹ = gC/newly produced cell). Carbon per NCC decreases upstream with decreasing salinity (Table 3 and Fig. 12). This trend occurs because TdR incorporation increases upstream, but leucine incorporation does not significantly change along the salinity gradient (Table 4). The amount of carbon being assimilated into protein is more constant, but the number of new cells is decreasing with increasing salinity downstream. This means that there is more carbon in the new cells downstream. Oddly enough, this occurs even though growth rate and average cell volume also decrease with increasing salinity. This indicates that the cells upstream may be more robust than those downstream and may be concentrating their metabolism on division rather than cell maintenance (Shiah and Ducklow 1998). Alternatively, if more cells are damaged or only semi-active (for whatever reason), those cells may be using carbon for sustenance and maintenance rather than growth.

The activity of a community on a per cell basis can be determined by using the ratio TdR/cell (Fig. 11). If all cells are active, TdR/cell may be interpreted as a measure of mean specific growth rate. Obviously, with the pattern of abundance to activity found in the York (Fig. 10), the TdR/cell ratio is much higher at station 6 than at station 1 (Fig. 11). This is especially true in the spring and fall when TdR/cell at station 6 is more than double the TdR/cell of any other station or time (Table 5). Why is abundance low when
growth rates are so high? The answer must be cell loss through predation, viral lysis or other removal process. If removal matched growth rate at station 6, cells would be removed as fast as they are created. With the large amounts of organic matter available (Fig. 2), conditions for growth are presumably favorable at station 6. Favorable conditions would likely lead to fewer inactive cells and thus a greater TdR/cell ratio.

High loss rates coupled with high TdR/cell would mean that TdR incorporation could be high even though cell abundance is relatively low. At station 1, where conditions are less favorable for growth, more cells may be inactive or less active, leading to a smaller TdR rate and thus to a lower TdR/cell ratio. In addition, if loss terms are not as large as at station 6, cells at station 1 would accumulate. It seems clear that for this pattern of inverse abundance and activity to persist in the York River estuary, removal must be a strong control on bacterial distribution along the salinity gradient (see Chapter 2).

**Bacterial vs phytoplankton production**

No significant relationship was seen between phytoplankton production or biomass (chlorophyll a) and the bacterial properties: abundance, TdR incorporation and leucine incorporation. This may indicate one of, or a combination of, several scenarios.

The relationship between bacterioplankton and phytoplankton may exist, but if phytoplankton material is in excess, bacterial properties may change without a corresponding change in phytoplankton properties. There may also be a temporal lag between phytoplankton production and bacterial use of the carbon produced.

Alternatively, there may be no relationship between the two sets of data.

Bacterioplankton of the York River may depend primarily upon allochthonous sources of organic matter for food substrates. Finally, there may be two or more different
communities of bacteria along the salinity gradient with different metabolic capabilities (see Chapter 3).

At first glance, the York system seems to have more than enough carbon produced by phytoplankton to support the bacterial carbon demand (BCD). Mean phytoplankton production over the sampling period was approximately 88 mgC-m⁻³-d⁻¹ at station 6, 372 mgC-m⁻³-d⁻¹ at station 5, and 326 mgC-m⁻³-d⁻¹ at station 1. The corresponding mean bacterial production rates were 51, 36 and 15 mgC-m⁻³-d⁻¹, respectively. However, these numbers are instantaneous measurements taken at the surface. They do not adequately describe the amount of carbon produced by phytoplankton or bacteria throughout the water column. Depending on the average depth of the euphotic zone and the water column, the ratios between phytoplankton and bacterial production may change dramatically.

Using measured values for the average water column depth at each station (Cronin, 1971), I obtained integrated production values for station 6, 5, and 1. Phytoplankton production was 123 mgC-m⁻²-d⁻¹ at station 6, 469 mgC-m⁻²-d⁻¹ at station 5 and 1000 mgC-m⁻²-d⁻¹ at station 1, while bacterial production is 186 mgC-m⁻²-d⁻¹, 93 mgC-m⁻²-d⁻¹, and 83 mgC-m⁻²-d⁻¹ at stations 6, 5 and 1 respectively (Fig.13). Integrated phytoplankton production is greater than integrated bacterial production at stations 5 and 1, but is smaller than BP at station 6.

Next, I interpolated over the entire estuary (see Methods for details) to obtain an estimate of phytoplankton and bacterial production for the entire estuary (Fig.14) for an entire year. Phytoplankton production is 4.3 x 10¹⁰ gC-year⁻¹, while bacterial production is 4.5 x 10⁹ gC-year⁻¹ for the York River estuary. Although primary production is an
Figure 13. Integrated production values at each of the three stations monitored by the EPA Bay Monitoring Program. Values are means of monthly data from the beginning of the sampling period (June 1996) through all months in which EPA phytoplankton production data were available as of January 1998. Bacterial production values were calculated from TdR-incorporation. PP – primary production, BP – bacterial production, BCD – bacterial carbon demand, BGE – bacterial growth efficiency.
Figure 14. Estimated carbon production by phytoplankton and bacteria in the York River estuary over a one year period. PP – primary production, BP – bacterial production, BCD – bacterial carbon demand, BGE – bacterial growth efficiency
Table 5. Bacterial production and growth rates grouped by season. Summer season includes June, July, and August; fall season includes September, October, and November. Winter season includes December, January, and February; while spring season includes March, April, and May. Values in parentheses are standard deviation of the mean.

<table>
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<th>Station</th>
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<th>Avg Temp (°C)</th>
<th>TdR (pM/l-hr)</th>
<th>Leu (pM/l-hr)</th>
<th>TdR/cell (pM/l-hr-cell) x 10^2</th>
<th>Leu/cell (pM/l-hr-cell) x 10^4</th>
<th>C/NCC (gC/new cell) x 10^-15</th>
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<tbody>
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<td>6 S</td>
<td>Summer</td>
<td>27.3 (1.0)</td>
<td>221 (52)</td>
<td>1105 (403)</td>
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<td>18.4 (15.3)</td>
<td>7.8 (2.4)</td>
</tr>
<tr>
<td>6 S</td>
<td>Fall</td>
<td>16.6 (6.7)</td>
<td>233 (225)</td>
<td>376 (195)</td>
<td>17.4 (9.0)</td>
<td>32.6 (20.1)</td>
<td>3.1 (2.1)</td>
</tr>
<tr>
<td>6 S</td>
<td>Winter</td>
<td>7.2 (1.5)</td>
<td>89 (79)</td>
<td>87 (11)</td>
<td>6.5 (2.9)</td>
<td>9.7 (5.5)</td>
<td>1.9 (0.8)</td>
</tr>
<tr>
<td>6 S</td>
<td>Spring</td>
<td>17.0 (3.0)</td>
<td>317 (315)</td>
<td>298 (166)</td>
<td>14.5 (15.9)</td>
<td>13.2 (7.4)</td>
<td>2.9 (1.8)</td>
</tr>
<tr>
<td>5 S</td>
<td>Summer</td>
<td>27.0 (0.7)</td>
<td>256 (80)</td>
<td>1461 (438)</td>
<td>3.8 (1.0)</td>
<td>15.7 (11.4)</td>
<td>9.1 (2.2)</td>
</tr>
<tr>
<td>5 S</td>
<td>Fall</td>
<td>16.5 (6.4)</td>
<td>130 (85)</td>
<td>388 (291)</td>
<td>7.6 (5.0)</td>
<td>19.8 (12.5)</td>
<td>4.6 (3.0)</td>
</tr>
<tr>
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<td>Winter</td>
<td>6.7 (1.0)</td>
<td>92 (79)</td>
<td>220 (247)</td>
<td>7.3 (6.9)</td>
<td>14.5 (16.7)</td>
<td>5.0 (4.4)</td>
</tr>
<tr>
<td>5 S</td>
<td>Spring</td>
<td>16.8 (3.2)</td>
<td>219 (179)</td>
<td>425 (182)</td>
<td>8.5 (7.7)</td>
<td>13.7 (6.5)</td>
<td>4.4 (2.5)</td>
</tr>
<tr>
<td>4 S</td>
<td>Summer</td>
<td>26.8 (0.6)</td>
<td>243 (93)</td>
<td>1367 (422)</td>
<td>4.2 (2.1)</td>
<td>10.7 (7.6)</td>
<td>9.0 (1.7)</td>
</tr>
<tr>
<td>4 S</td>
<td>Fall</td>
<td>16.3 (6.3)</td>
<td>104 (91)</td>
<td>358 (310)</td>
<td>3.5 (3.5)</td>
<td>12.0 (11.7)</td>
<td>5.5 (1.5)</td>
</tr>
<tr>
<td>4 S</td>
<td>Winter</td>
<td>6.7 (1.2)</td>
<td>32 (9)</td>
<td>139 (96)</td>
<td>1.7 (0.5)</td>
<td>6.7 (3.2)</td>
<td>6.2 (2.8)</td>
</tr>
<tr>
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<td>16.5 (3.6)</td>
<td>187 (122)</td>
<td>521 (184)</td>
<td>4.2 (4.2)</td>
<td>13.5 (4.4)</td>
<td>6.1 (3.0)</td>
</tr>
<tr>
<td>3 S</td>
<td>Summer</td>
<td>26.2 (0.8)</td>
<td>191 (49)</td>
<td>1330 (234)</td>
<td>2.7 (0.5)</td>
<td>10.8 (7.1)</td>
<td>4.1 (1.6)</td>
</tr>
<tr>
<td>3 S</td>
<td>Fall</td>
<td>16.5 (6.3)</td>
<td>103 (70)</td>
<td>404 (324)</td>
<td>3.4 (3.3)</td>
<td>11.3 (10.5)</td>
<td>5.7 (1.8)</td>
</tr>
<tr>
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<td>6.4 (1.2)</td>
<td>25 (4)</td>
<td>210 (205)</td>
<td>1.3 (0.5)</td>
<td>8.5 (5.1)</td>
<td>11.7 (1.6)</td>
</tr>
<tr>
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<td>159 (89)</td>
<td>482 (138)</td>
<td>2.9 (2.7)</td>
<td>13.4 (5.6)</td>
<td>5.9 (2.4)</td>
</tr>
<tr>
<td>2 S</td>
<td>Summer</td>
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<td>142 (52)</td>
<td>989 (40)</td>
<td>1.8 (1.0)</td>
<td>11.2 (10.7)</td>
<td>12.1 (3.7)</td>
</tr>
<tr>
<td>2 S</td>
<td>Fall</td>
<td>16.8 (6.4)</td>
<td>77 (60)</td>
<td>433 (346)</td>
<td>2.5 (2.5)</td>
<td>11.6 (12.0)</td>
<td>8.2 (3.4)</td>
</tr>
<tr>
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<td>6.3 (0.9)</td>
<td>28 (9)</td>
<td>198 (230)</td>
<td>1.1 (0.3)</td>
<td>6.2 (4.8)</td>
<td>8.7 (7.4)</td>
</tr>
<tr>
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<td>Spring</td>
<td>15.6 (4.0)</td>
<td>135 (105)</td>
<td>603 (313)</td>
<td>3.0 (2.7)</td>
<td>12.9 (11.3)</td>
<td>9.5 (3.7)</td>
</tr>
<tr>
<td>1 S</td>
<td>Summer</td>
<td>25.4 (1.4)</td>
<td>113 (46)</td>
<td>1139 (518)</td>
<td>1.3 (0.7)</td>
<td>8.5 (8.0)</td>
<td>15.4 (3.4)</td>
</tr>
<tr>
<td>1 S</td>
<td>Fall</td>
<td>16.5 (6.2)</td>
<td>66 (55)</td>
<td>415 (340)</td>
<td>1.4 (1.0)</td>
<td>9.4 (9.9)</td>
<td>9.8 (4.2)</td>
</tr>
<tr>
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<td>Winter</td>
<td>6.5 (2.2)</td>
<td>24 (5)</td>
<td>87 (58)</td>
<td>1.0 (0.4)</td>
<td>4.9 (3.2)</td>
<td>5.8 (2.8)</td>
</tr>
<tr>
<td>1 S</td>
<td>Spring</td>
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<td>99 (71)</td>
<td>456 (160)</td>
<td>2.7 (2.4)</td>
<td>10.8 (10.3)</td>
<td>9.0 (2.9)</td>
</tr>
</tbody>
</table>
order of magnitude greater than bacterial production, the bacterial growth efficiency (BGE = BP/BCD) must still be considered to determine if the phytoplankton production provides enough carbon to meet the entire bacterial carbon demand.

For the York River bacterioplankton community to be independent of the need for allochthonous carbon sources, BGE must be greater than 10.4% over the entire estuary. Measurements of the BGE of marine bacteria range from 2 to 55% (Griffith et al. 1990, Kirchman et al. 1991, Coffin et al. 1993, Biddanda et al. 1994). Biddanda et al. (1994) found bacterial growth efficiencies of 26% in the slope waters and 50% in the more productive shelf waters of the Gulf of Mexico. Using a BGE of 10%, the phytoplankton production in the entire York River was about equal to BCD. However, if we assume a BGE of 20%, then there was about $2 \times 10^{13}$ mgC-yr$^{-1}$ extra carbon produced (Fig. 13). If the BGE is closer to Biddanda et al.'s (1994) value of 50%, then there would be about $3.4 \times 10^{13}$ mgC-yr$^{-1}$ excess carbon produced in the York (Fig. 14). This excess carbon is available for burial or export to the Chesapeake Bay.

This large amount of excess carbon estimated to be produced in the York River includes the effects of station 6. The freshwater region (station 6) of the York River located in the Pamunkey River is different from the rest of the estuary. At station 6, the Pamunkey River is very turbid and relatively deep. Phytoplankton production in this area is unlikely to be able to meet bacterial carbon demand. Over the course of this study, BP:PP at station 6 was approximately 1.5. Thus, at station 6, bacteria must utilize allochthonous substrates to meet their carbon demand.

Other systems with relatively large BP:PP ratios have been found in light limited systems that have large allochthonous inputs. Bano et al. (1997) reported BP:PP ratios
greater than one in the tidal creeks of a mangrove system. Goosen et al. (1997) also
found high BP:PP ratios in the highly turbid Schelde estuary (SW Netherlands). Of
course, these estimates of BP:PP are highly dependent upon the conversion factors used
in determining bacterial production from TdR incorporation and upon the actual bacterial
growth efficiency, which is still poorly known and difficult to measure. However, it does
appear as though the York River estuary produced more carbon than could be consumed
by heterotrophic bacteria over the course of this study.
CHAPTER 2

CIRCULATION AND BACTERIAL GROWTH DYNAMICS IN A SMALL ESTUARY: YORK RIVER, VA
INTRODUCTION

Bacterial dynamics in estuaries have been well-studied (Ducklow and Shiah 1993). However, few studies have included the effects of physical processes such as estuarine circulation, on bacterial distribution. Because pelagic bacteria are planktonic, their distribution is principally controlled by water circulation. Other factors such as growth and grazing may control their overall abundance and production, but where the bacteria are located as these processes take place is completely controlled by water movement. If water movement is slow or stagnant, bacteria may remain in one region for periods of time long enough for biological effects to control their distribution. If this is the case, models that ignore circulation while depending upon top-down effects (grazing) or bottom-up effects (temperature and substrate) are more applicable for describing bacterial distributions (Wright 1988, Billen et al. 1990). While this may be appropriate in some systems, water movement is rarely stagnant in estuaries. Freshwater inflow, tidal currents, and wind interact to create complex patterns of water movement. If water movement is swift, bacteria may not stay in a particular region of an estuary long enough for biological processes to predominate in that region. Thus it is important to determine rates of physical mixing and advection as well as biological processes to obtain a complete picture of bacterial dynamics in an estuarine system.
Ketchum (1954) first noted that plankton distribution is strongly affected by circulation. His study on coliform bacteria in the Raritan River (Ketchum et al. 1952) was the first to include physical processes in a study of estuarine bacteria. Although there have been a few studies that demonstrated the importance of physical processes such as wind on bacterial dynamics (Legendre and Troussellier 1988, Troussellier et al. 1993), until Painchaud (1987), there was no general model of estuarine bacterial dynamics that included water circulation. Painchaud (1987) used a simple box model (Officer 1980) to model the distribution of bacteria in the Saint Lawrence estuary. This study examined only circulation effects on bacteria in the Saint Lawrence estuary and did not include information on biological processes (i.e. growth and removal) affecting bacterial distribution. To rectify this situation, Painchaud et al. (1996) again investigated the Saint Lawrence. In this study, biological processes were integrated into their model to obtain a more complete picture of the bacterial dynamics of the Saint Lawrence estuary over short time scales (days).

The Officer (1980) model uses salinity, river discharge, and bacterial abundance to obtain hydrodynamic residence time, dispersion rate and bacterial fluxes. The hydrodynamic residence time is defined as the average lifetime of a conservative particle in a given volume of the estuary (Craig 1957). Hydrodynamic residence time in a particular volume of the estuary is given by the quotient of the steady state mass of a conservative element and the flux into or out of the volume. The residence time is important in consideration of the processes that control the distribution of an estuarine material. If residence time is small relative to biological, physical, chemical or even geological transformations that a material is undergoing, then little will happen to the
material in the volume in question. If however, the residence time is long, the reaction may be completed within the volume.

Net bacterial fluxes can be found using the box model approach. Net fluxes so determined include both physical and biological processes acting upon estuarine bacteria. Using these net fluxes with observed bacterial abundance and production can provide estimates of growth and grazing rates. Examining hydrodynamic residence time, dispersion rates and bacterial fluxes allows us to compare the relative importance of biological and physical processes in the estuarine system.

Painchaud et al's (1996) work in the Saint Lawrence inspired me to attempt to model the York River estuary in the same manner. I used monthly observations in the York River to attempt to understand how physical and biological processes interact over a seasonal scale. The York River is weakly stratified, with periodic destratification after the monthly spring tide. This destratification forced us to use a one-dimensional model (Officer 1980), as well as the two-dimensional model employed by Painchaud (1996).

**MATERIALS AND METHODS**

**Study site**

The York River is a sub-estuary of the Chesapeake Bay with a watershed that lies between those of the James and the Rappahannock Rivers. The York flows in a southeasterly direction 47.5 km from the confluence of the Pamunkey and Mattaponi Rivers at West Point, Virginia to the mouth near Yorktown, Virginia. The average depth of the York ranges from ≥ 7 m at the mouth to ≤ 3 m at West Point. The entire sampling
area is tidally influenced with a mean tidal range of 0.61 m at the mouth of the York, increasing to 0.88 m at West Point before decreasing again (Cronin 1971). Brackish water extends approximately 60 kilometers upstream in the York River estuary. The average annual freshwater discharge rate was approximately 50 m$^3$-s$^{-1}$ during the sampling period (USGS 1999).

Sample collection

Samples were collected monthly from 1 m below the surface and from 1 m above the bottom, at six stations along the salinity gradient of the York River (Fig. 1) aboard the RV 'Kingfisher' during cruises from June 1996 through May 1997. Stations were approximately 9 km apart with Station 1 located at the mouth of the York and Station 6 located in freshwater in the Pamunkey River approximately 60 kilometers from the mouth. Samples were collected at each station using a clean 2.5-liter Niskin bottle. Water was collected for determination of bacterial cell abundance, bacterial production, temperature and salinity.

Bacterial cell abundance and biovolume measurements

Samples for bacterial abundance and biovolume were preserved with 0.2 μm filtered 25% glutaraldehyde (Sigma) diluted to a final concentration of 2% glutaraldehyde. Preserved samples were then stored at 4°C until slide preparation. Slide preparation occurred within seven days of collection. Two (2) to six (6) ml of sample were filtered onto 0.2 μm black polycarbonate filters (Poretics). A mixed-ester backing filter (MSI) was used to ensure even distribution of cells on the slide. As the samples were filtered, 200 ml of acridine orange (Sigma) solution (final concentration 0.005%)
Figure 1. Map of site locations (stations 1 – 6). York River estuary, VA. Box volumes were created by separating the river approximately mid-way between each station.
were added to stain the cells for viewing (Hobbie et al., 1977). Once the sample had completely filtered, but before the filter could become dry, the black polycarbonate filter was removed from the filter housing and mounted on a glass slide in Resolve immersion oil and frozen until examination.

All slides were enumerated by epifluorescence microscopy. A Zeiss Axiophot microscope at 1613 x with a blue BP 450 – 490 excitation filter and an LP-520 barrier filter was used. To estimate biovolume, images were taken with a Dage-MTI Nuvicon video camera connected to the Axiophot microscope through a Dage Gen-II image intensifier. Images were processed and analyzed using a PC 486-based Zeiss Vidas Videoplan Image Analysis system. Fluorescent spheres of various sizes (Polysciences Corp.) were used for calibration. Cell volumes were estimated using the algorithm of Baldwin and Bankston (1988).

**Bacterial production – ^3^H-thymidine incorporation**

Bacterial production was estimated from [^3^H-methyl]-thymidine (^3^H-TdR) incorporation. ^3^H-TdR (Dupont, specific activity ≈ 80 Ci/mmol) was added to forty-eight 2.0 µl centrifuge tubes. The concentration of ^3^H-TdR was such that 10 µl of isotope solution with 1.7 ml of sample gave a final concentration of 25 nM Thymidine. At each station, four tubes with ^3^H-TdR were used. One of the four tubes (of each treatment) was made a blank by the addition of 100µl of ice-cold 100% trichloroacetic acid (TCA; Fisher) prior to addition of sample. These tubes were pre-filled with isotope solution and taken out on sampling cruises on ice. Once water was collected, 1.7 ml of sample was added to four tubes with ^3^H-TdR (three replicates and a blank). Samples were inverted to mix and then placed into water held at near *in situ* temperature (± 2.0° C). Samples were
allowed to incubate approximately one hour, at which time the incubations were stopped by adding 100 μl of 100% TCA to the centrifuge tube and gently mixing. Samples were then placed on ice until return to the lab. Samples were processed immediately after return to the lab. Processing consisted of spinning the samples in a high-speed centrifuge (Eppendorf, model 5402) at 14,000 rpm for seven minutes at 2° C to create a pellet containing bacterial cell walls and molecules too large to pass through the membranes compromised by the TCA addition (namely, DNA and proteins). At that time, the supernatant was aspirated and 1.8 ml of 5\% ice cold TCA was added. The samples were spun again for seven minutes and again the supernatant was aspirated. This time, 1.8 ml of 80\% ethanol (Fisher) was added. The samples were spun a final time followed by aspiration of the supernatant. One ml of Ultima Gold scintillation cocktail (Packard) was added and the radioactivity of the samples was counted by a Wallac Liquic Scintillation Counter.

**Conversion factors**

To obtain bacterial carbon values from incorporation of TdR, a carbon conversion factor was used. For this study, the midrange carbon conversion factor of 120 fg C μm\(^{-3}\) (Watson *et al.* 1977) was selected. A volumetric factor was chosen to ensure that differences in bacterial cell size were taken into account. Thus, bacterial carbon values were calculated from bacterial cell abundance and average cell volume for each sample. To calculate bacterial cell production, an additional conversion factor was used. In this case, I used the conversion factor of 1.1 X 10\(^{18}\) cells/mole of thymidine incorporated determined for an estuary (Riemann *et al* 1987).
Model calculations – flux calculations

Conservative properties in an estuary are controlled by circulation. A partially mixed estuary is characterized by a net seaward flow at the surface, a net landward flow at depth, and a net vertical flux toward the surface. Using a simple box model described by Officer (1980), water and property fluxes may be calculated. These fluxes can be used to determine if the distributions of dissolved or particulate materials in the estuary are completely controlled by circulation. This box model also allows regions of the estuary to be identified as sources or sinks of the component in question. Using this approach, residence times for the component of interest within a given volume of the estuary may be determined. Finally, rates of production and loss may be back-calculated as well.

Flux calculations are based on the conservative nature of salinity distributions. Because this model assumes steady state conditions, monthly sampling cruises were each modeled according to the state of the estuary at that time. If the estuary was well-mixed, a one-dimensional box model was used. If the estuary was stratified, a two-dimensional box model was used.

One-dimensional box model

To model the distribution of bacterial cells in the York during times of destratification, a one-dimensional box model was used. The estuary was divided into 6 approximately equal regions surrounding each of the six sampling stations (Fig. 1). Figure 2 shows the notation system and calculations to be used for the one-dimensional box model. Note that the sampling stations were numbered from the mouth to freshwater, while, for purposes of the model, the boxes are numbered from freshwater to
Figure 2. Equations and symbols for one-dimensional box model.
\[ E_{m,m+1} = E_{m+1,m} = \frac{S_m R}{(S_{m+1} - S_m)} \]

\[ E_{m-1,m} = E_{m,m-1} = \frac{S_{m-1} R}{(S_m - S_{m-1})} \]

\[ \tau_m = \frac{(S_m - S_{m-1}) (S_{m+1} - S_m)}{S_m (S_{m+1} - S_{m-1})} \frac{V_m}{R} \]
the mouth. The boundaries of the boxes are the water surface, the estuary bottom and vertical sections between each station perpendicular to the advective flow. All observations of bacterial cell abundance and salinity were averages of surface and bottom measurements. The box volumes need not be equally sized (and are not here). River flow ($R$, m$^3$sec$^{-1}$) was measured by the United States Geological Survey (USGS) at the fall line of the Pamunkey and Mattaponi Rivers. For purposes of consistency with Sin et al. (1999), only discharge data from the Pamunkey was used. The Pamunkey accounts for $\sim 60 - 70$% of the freshwater discharge into the York (USGS 1999).

$E$ is the non-advective exchange coefficient between each segment. It includes both tidal exchange and net circulation. Exchange coefficients between boxes were determined by differences in salinity using the continuity equation, which states that the sum of fluxes into a box equals the sum of fluxes leaving the box. Water transport into and out of each box were then calculated by using the product of the river discharge and the exchange coefficients. Measured bacterial cell abundance from the single fixed station within each volume was then multiplied by the water transport to obtain the bacterial exchange rates. Summation of the calculated transports into and out of a box yields the net bacterial transport in cells-hour$^{-1}$ for each segment of the river. If these net exchange rates are not zero, then that volume of water demonstrates either production or loss of bacterial cells due to processes other than circulation. Negative net exchange indicates loss of cells within a box while positive net exchange indicates a source of cells within a box. To be able to compare these exchange rates against measured values of production or loss, the exchange rates were divided by the volume of each box to obtain fluxes in terms of cells-volume$^{-1}$-time$^{-1}$.
Two-dimensional box model

During most of the year, the York River is weakly stratified. Generally, flow in a stratified or partially stratified estuary is down-estuary at the surface and up-estuary at depth. To account for this difference in flow, Officer's (1980) two-dimensional box model must be employed. The horizontal division divides the box $m$ into an upper and lower box. This division corresponds to the division of the water column at the halocline. Salinity values below the halocline are denoted as $S_m'$. By dividing the boxes vertically, the effects of tidal exchange and net circulation can be separated. This is important because tidal exchange is essentially depth independent while net circulation is essentially depth dependent in a stratified system. Figure 3 shows the notation system and calculations used for the two-dimensional box model.

An important difference between one-dimensional and two-dimensional models is the use of the non-dimensional diffusivity constant $\nu (0 \leq \nu \leq 1)$ in two-dimensional models (Fig. 3). This constant is the ratio of the salt diffusional exchange to the total longitudinal dispersion (Hansen and Rattray 1966, Dyer 1973). If $\nu = 1$, there is no gravitational circulation and upstream salt flux is completely by diffusion. If $\nu = 0$, then upstream salt flux is almost entirely due to gravitational circulation and diffusion is unimportant. Therefore, large rivers with strong gravitational circulation will have diffusivity constants below 0.5 while smaller rivers with weaker gravitational circulation will have diffusivity constants greater than 0.5. The York is weakly stratified with monthly periods of destratification (Haas 1977). Therefore, for my model, I used a value for $\nu$ of 0.7. This also maintains consistency with Painchaud (1995) and allows easy comparisons of fluxes.
Figure 3. Equations and symbols for two-dimensional box model.
\[ Q_m = \frac{R(S_m - S_{m-1})}{S_m - S_{m-1}} \]
Calculations for hydrodynamic residence time

Hydrodynamic residence time is the average life of a conservative component in a given volume of the estuary. Residence time was calculated as the ratio of the volume of a box to the horizontal and vertical fluxes of water out of a box (see Figs. 2 & 3).

RESULTS

Precision and Sensitivity

The model was tested for computational errors by calculating salinity fluxes and ensuring that net salinity fluxes were zero. To estimate the precision of the flux values, Painchaud et al. (1996) varied $\nu$ by 0.1. I used the same method to determine the precision of the model (Table 1). This variation did not change the pattern of fluxes in the estuary. With $\nu = 0.8$, volumetric fluxes were slightly larger and with $\nu = 0.6$, volumetric fluxes were slightly lower. The mean coefficient of variation thus found averaged 19%, essentially equal to the adjustment of $\nu$ (14%).

The sensitivity of the model depends on the variability of the relevant inputs (freshwater discharge, salinity, bacterial concentration, and diffusivity fraction). The freshwater discharge may change over a scale of days, while the salinity and bacterial concentrations at a given point in the river may change over a scale of hours. Therefore, these estimates of fluxes must be considered to be "snapshots" of the estuary at the given time. However, since these "snapshots" were taken over an annual period, they should
Table 1. Monthly calculations of net volumetric bacterial fluxes for each station from June 1996 through May 1997. $v = 0.7$. Values are $10^6$ bacteria-l^{-1}-hr^{-1}. Numbers in parentheses result from varying $v$ by ± 0.1 (i.e. by 14%) and are analogous to standard deviation. If the estuary was mixed, $v$ was equal to one.

<table>
<thead>
<tr>
<th>Date</th>
<th>Station 5</th>
<th>Station 4</th>
<th>Station 3</th>
<th>Station 2</th>
</tr>
</thead>
<tbody>
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<td>June 1996</td>
<td>Surface</td>
<td>16.1 (±34%)</td>
<td>-6.7 (±37%)</td>
<td>-13.7 (±10%)</td>
</tr>
<tr>
<td>June 1996</td>
<td>Bottom</td>
<td>-21.2 (±18%)</td>
<td>16.3 (±6%)</td>
<td>-5.9 (±12%)</td>
</tr>
<tr>
<td>July 1996</td>
<td>Surface</td>
<td>-5.1</td>
<td>13.6</td>
<td>-22.0</td>
</tr>
<tr>
<td>July 1996</td>
<td>Bottom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 1996</td>
<td>Surface</td>
<td>2.8</td>
<td>-1.7</td>
<td>3.0</td>
</tr>
<tr>
<td>August 1996</td>
<td>Bottom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>September 1996</td>
<td>Surface</td>
<td>0.2 (±50%)</td>
<td>2.4 (±29%)</td>
<td>1.2 (±8%)</td>
</tr>
<tr>
<td>September 1996</td>
<td>Bottom</td>
<td>6.1 (±10%)</td>
<td>-7.2 (±1%)</td>
<td>5.3 (±9%)</td>
</tr>
<tr>
<td>October 1996</td>
<td>Surface</td>
<td>-2.5 (±12%)</td>
<td>1.0 (±60%)</td>
<td>11.8 (±3%)</td>
</tr>
<tr>
<td>October 1996</td>
<td>Bottom</td>
<td>-9.8 (±5%)</td>
<td>9.0 (±85)</td>
<td>3.8 (±18%)</td>
</tr>
<tr>
<td>November 1996</td>
<td>Surface</td>
<td>-8.3</td>
<td>9.2</td>
<td>3.2</td>
</tr>
<tr>
<td>November 1996</td>
<td>Bottom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>December 1996</td>
<td>Surface</td>
<td>-15.1 (±9%)</td>
<td>20.7 (±30%)</td>
<td>-7.0 (±27%)</td>
</tr>
<tr>
<td>December 1996</td>
<td>Bottom</td>
<td>3.4 (±3%)</td>
<td>-18.4 (±29%)</td>
<td>5.8 (±10%)</td>
</tr>
<tr>
<td>January 1997</td>
<td>Surface</td>
<td>-10.9 (±5%)</td>
<td>0.5 (±20%)</td>
<td>-0.4 (±50%)</td>
</tr>
<tr>
<td>January 1997</td>
<td>Bottom</td>
<td>-2.3 (±9%)</td>
<td>0.4 (±25%)</td>
<td>-2.2 (±5%)</td>
</tr>
<tr>
<td>February 1997</td>
<td>Surface</td>
<td>-35.9 (±8%)</td>
<td>2.0 (±5%)</td>
<td>4.0 (±15%)</td>
</tr>
<tr>
<td>February 1997</td>
<td>Bottom</td>
<td>0.2 (±50%)</td>
<td>2.0 (±5%)</td>
<td>-1.6 (±13%)</td>
</tr>
<tr>
<td>March 1997</td>
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<td>2.1</td>
<td>-6.3</td>
</tr>
<tr>
<td>March 1997</td>
<td>Bottom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 1997</td>
<td>Surface</td>
<td>-3.5 (±23%)</td>
<td>-1.4 (±7%)</td>
<td>-1.6 (±44%)</td>
</tr>
<tr>
<td>April 1997</td>
<td>Bottom</td>
<td>2.8 (±18%)</td>
<td>-0.2 (±50%)</td>
<td>-2.7 (±44%)</td>
</tr>
<tr>
<td>May 1997</td>
<td>Surface</td>
<td>2.8</td>
<td>15.2</td>
<td>-16.5</td>
</tr>
<tr>
<td>May 1997</td>
<td>Bottom</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
provide a representative picture of the upper and lower bounds of bacterial fluxes within the York River estuary.

**Bacterial properties, temperature and salinity**

From June 1996 to May 1997, I sampled the York at 6 stations along the salinity gradient as part of a larger study on bacterial dynamics (see Ch. 1). During the study period, salinity in the York River ranged from < 1.0 °/oo at station 6 to as high as 17.5 °/oo at station 1. Salinity at each station was higher in the summer months than in the winter months. Water temperature varied little along the salinity gradient on any given date. Over the course of the year, water temperature ranged from ~ 5°C in the winter to greater than 25°C in the summer. In every sampling period, cell abundance increased with increasing salinity while 3TdR-incorporation decreased with increasing salinity (Figs. 4 and 5; see Ch. 1 for details). Cell abundance and 3TdR-incorporation at depth generally followed the patterns of surface abundance and incorporation, respectively.

Bacterial cell abundance showed a distinct seasonal pattern (Fig. 4). Cell abundance was greatest in the summer months, and fell during the fall to minimum values in the late fall or winter before rising again in the spring. 3TdR-incorporation also showed a similar seasonal pattern (Fig. 5). Incorporation rates were greatest in warm water months and lowest during periods of temperature minima.

**Model Results - Bacterial exchange rates**

Bacterial exchange rates in the York River were determined for each month of the sampling period (Fig. 6). Because CTD data were not available, the mixed status of the estuary was determined by difference in salinity between surface and bottom waters.
Figure 4. Contour plot of surface bacterial cell abundance at all stations measured monthly from June 1996 (labelled on x axis as 1) to May 1997 (labelled on x-axis as 12). Abundance values are $x 10^9$ cells-liter$^{-1}$. Station 1 is at the mouth of the York, while station 6 is on the Pamunkey River.
Figure 5. Contour plot of surface bacterial production (from TdR incorporation) at all stations measured monthly from June 1996 (labelled on x axis as 1) to May 1997 (labelled on x-axis as 12). Production values are mgC-m$^{-3}$-day$^{-1}$. Station 1 is at the mouth of the York, while station 6 is on the Pamunkey River.
Figure 6. Box model showing bacterial exchange rates in the York River from June 1996 through November 1996. Bacterial exchange rates are $x \times 10^{17}$ cells-hr$^{-1}$. Numbers in boxes are rates of production (+) or loss (-).
<table>
<thead>
<tr>
<th>Station 5</th>
<th>Station 4</th>
<th>Station 3</th>
<th>Station 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0.7</td>
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<td></td>
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<td>7.1</td>
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<td>4.9</td>
<td>-2.9</td>
<td>6.7</td>
<td>-9.2</td>
</tr>
<tr>
<td>43</td>
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<td>-14.8</td>
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<td>21.7</td>
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<td>-3.1</td>
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<tr>
<td>-2.6</td>
<td>2.0</td>
<td>2.1</td>
<td>12.6</td>
</tr>
</tbody>
</table>

**June 17, 1996**

**July 16, 1996**

**August 19, 1996**

**September 17, 1996**

**October 24, 1996**

**November 19, 1996**

*Freshwater Bacterial Fluxes (x10^7 cells/hr)*

*River Mouth*
Figure 6 (cont.). Box model showing bacterial exchange rates in the York River from December 1996 through May 1997. Bacterial exchange rates are $10^{17}$ cells-hr$^{-1}$. Numbers in boxes are rates of production (+) or loss (-).
<table>
<thead>
<tr>
<th>Date</th>
<th>Station 5</th>
<th>Station 4</th>
<th>Station 3</th>
<th>Station 2</th>
</tr>
</thead>
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<tr>
<td>December 16, 1996</td>
<td>-4.6 4.2</td>
<td>9.0 11.0</td>
<td>-4.7 6.9</td>
<td>20.7 29.3</td>
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<td>January 22, 1997</td>
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<td>0.3 2.8</td>
<td>-6.8 0.4</td>
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<tr>
<td>February 18, 1997</td>
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<td>-2.7 2.3</td>
<td>5.6 7.8</td>
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<td>6.8 12.2</td>
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<td>April 15, 1997</td>
<td>1.6 2.4</td>
<td>6.6 9.0</td>
<td>-11.0 2.0</td>
<td>0.3 1.7</td>
</tr>
</tbody>
</table>

**Freshwater Bacterial Fluxes (x10^7 cells/hr)**
Each station was considered stratified if the surface salinity was more than 1.0 °/oo lower than the bottom salinity at that station. Exceptions occurred during October 1996 when the difference was 0.9 °/oo at station 4, December 1996 when surface salinity at station 4 was 0.4 °/oo lower than the bottom salinity, and during February 1997 when the difference was 0.3 °/oo at station 5. In all three cases, the difference in salinity at all other stations was > 1 °/oo. If the difference between surface salinity and bottom salinity was less than 1.0 °/oo at the majority of the stations, the estuary was considered well-mixed and a one-dimensional box model was used. This occurred in July 1996, March 1997 and May 1997. A two-dimensional box model was used for June, September, October and December 1996, and January, February, and April 1997. In August and November 1996, the York was stratified at station 2 but mixed upstream. In these months, a one-dimensional box model was used for stations 5 - 3 and fluxes for station 2 were determined using a two-dimensional model with v = 1 so that the model would balance. When the estuary was well mixed, upstream salt flux was due entirely to diffusion and v was set to one. For all two-dimensional models v was set to 0.7.

Exchange rates from month to month were quite variable. In June 1996, horizontal bacterial exchange rates were generally larger than vertical exchange rates (Fig. 6). The largest transport of cells was out of station 2 into the mouth of the estuary. Stations 2S (surface) and 4B (bottom) had the largest production rates. In contrast, stations 5B and 3S had the largest loss rates. The estuary was well mixed in July 1996. Exchange rates increased with distance downstream. Station 2 had the largest production rate while station 3 had the largest loss rate in July 1996. Again, the largest transport was to the mouth of the York (Fig. 6).
Table 2. Residence times in days for each box volume for each month studied.

<table>
<thead>
<tr>
<th>Month</th>
<th>Depth</th>
<th>Station 5</th>
<th>Station 4</th>
<th>Station 3</th>
<th>Station 2</th>
<th>Total</th>
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<td>4.75</td>
<td>6.45</td>
<td>9.25</td>
<td>27.25</td>
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<td>7.19</td>
<td>5.43</td>
<td>8.03</td>
<td>10.97</td>
<td>31.61</td>
</tr>
<tr>
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<td>Surface</td>
<td>5.90</td>
<td>3.88</td>
<td>3.94</td>
<td>4.35</td>
<td>18.08</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>August</td>
<td>Surface</td>
<td>15.75</td>
<td>9.75</td>
<td>6.72</td>
<td>5.08</td>
<td>37.30</td>
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<td></td>
<td></td>
<td>7.52</td>
<td>7.52</td>
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<tr>
<td>September</td>
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<td>16.83</td>
<td>9.28</td>
<td>10.70</td>
<td>10.31</td>
<td>47.12</td>
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<td>Bottom</td>
<td>16.55</td>
<td>9.59</td>
<td>6.38</td>
<td>6.51</td>
<td>39.02</td>
</tr>
<tr>
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<td>Surface</td>
<td>7.34</td>
<td>2.64</td>
<td>4.56</td>
<td>3.69</td>
<td>18.23</td>
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<tr>
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<td>Bottom</td>
<td>7.18</td>
<td>2.79</td>
<td>4.72</td>
<td>0.62</td>
<td>15.31</td>
</tr>
<tr>
<td>November</td>
<td>Surface</td>
<td>7.12</td>
<td>4.71</td>
<td>4.22</td>
<td>3.49</td>
<td>19.54</td>
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<td>Bottom</td>
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<td></td>
<td></td>
<td>5.23</td>
<td>5.23</td>
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<tr>
<td>December</td>
<td>Surface</td>
<td>3.32</td>
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<td>3.88</td>
<td>1.04</td>
<td>3.14</td>
<td>1.94</td>
<td>10.00</td>
</tr>
<tr>
<td>January</td>
<td>Surface</td>
<td>4.31</td>
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<td>2.39</td>
<td>14.35</td>
</tr>
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<td>Bottom</td>
<td>4.75</td>
<td>3.65</td>
<td>4.37</td>
<td>2.09</td>
<td>14.85</td>
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<td>February</td>
<td>Surface</td>
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<td>2.04</td>
<td>2.10</td>
<td>8.72</td>
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<td>Bottom</td>
<td>2.54</td>
<td>2.79</td>
<td>2.28</td>
<td>1.90</td>
<td>9.51</td>
</tr>
<tr>
<td>March</td>
<td>Surface</td>
<td>5.26</td>
<td>2.94</td>
<td>2.87</td>
<td>1.44</td>
<td>12.52</td>
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</tr>
<tr>
<td>April</td>
<td>Surface</td>
<td>4.04</td>
<td>5.35</td>
<td>5.15</td>
<td>7.20</td>
<td>21.73</td>
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<td>4.83</td>
<td>3.48</td>
<td>17.92</td>
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<td>Surface</td>
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<td>4.30</td>
<td>3.95</td>
<td>4.23</td>
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<td>Average</td>
<td>Surface</td>
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<td>4.54</td>
<td>4.75</td>
<td>4.72</td>
<td>21.20</td>
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<td>7.41</td>
<td>4.61</td>
<td>4.62</td>
<td>3.84</td>
<td>20.49</td>
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</table>
In August 1996, exchange rates were low throughout the mixed regions of the estuary. However, in station 2, both vertical and horizontal exchange rates were relatively large, with the largest transport rate out of the surface box and out to the bay. Overall, the estuary produced cells with only station 4 showing a small loss rate (Fig. 6). Vertical exchange rates in September 1996 were again generally lower than horizontal exchange rates. Net exchange rates were relatively small throughout the estuary (Fig. 6).

October 1996 exhibited a large exchange rate of cells into the estuary from the mouth into the bottom waters of station 2. The cells were lost here and in the surface of station 2 as shown by the large negative loss rates. November 1996 was similar to August 1996 with low exchange rates through all stations except the stratified station 2. Stations 4 and 3 exhibited produced cells during November 1997. These cells are transported downstream and out to the mouth or lost in station 5 (Fig. 6).

Horizontal exchange rates were relatively high throughout the estuary in December 1996, with the highest exchange rates being out of the estuary from both the surface and bottom layers of station 2. Production occurred at all stations except for station 5S and station 3S. Thus, the estuary as a whole produced cells during December (Fig. 6). Exchange rates during January 1997 were relatively small with most being less than $3 \times 10^{17}$ cells/hr. There was no export of cells to the mouth in this during January 1997 (Fig. 6). Exchange rates in February 1997 were also relatively small. However, there was a large transport rate of cells into the estuary at station 5S. Most of these cells were consumed there as indicated by the loss rate. Vertical exchange rates during February are much lower than the horizontal exchange rates. The largest production rate was at station 2S. This provided the cells that were exported to the mouth (Fig. 6).
The estuary was well mixed during March 1997. At this time, the estuary exhibited loss rates at all stations except station 4 where there was a small production of cells. There was also a transport of cells into the estuary from the mouth (Fig. 6). In April 1997 the York was again stratified. Loss rates in stations 5, 4 and 3 were small, but station 2 had a relatively large production rate in both the surface and bottom layers. There was an export of cells to the mouth from both layers of station 2 (Fig. 6). Finally, May 1997 resembled March 1997. The estuary was well mixed and had a transport of cells into the estuary from the mouth. However, unlike March, stations 3 and 4 had relatively large loss and production rates, respectively. Station 4 was a source of cells, while station 3 was the only sink for cells in the estuary (Fig. 6).

Model results - volumetric bacterial fluxes

Volumetric bacterial fluxes were determined by dividing the bacterial production or loss rates by the mean volume of their particular box (Fig. 7). This gave a term with units of cells·l⁻¹·hr⁻¹ and allowed us to compare the fluxes in each reach of the estuary. Volumetric fluxes were variable at each station with no clear seasonal signals (Fig. 7). At the surface, station 5 was generally a sink for bacteria with 9 of 12 months showing a negative volumetric flux. Station 3 was also usually a sink for bacteria with 8 of 12 months showing a negative volumetric flux. Stations 4 and 2, however, were sources for bacteria 9 of 12 and 7 of 12 months, respectively (Fig. 7). Similar patterns were observed at depth (Fig. 7).
Figure 7. Monthly net volumetric bacterial fluxes. Values are $x 10^6$ cells-liter$^{-1}$-hour$^{-1}$. Number in top right of each box indicates station number as well as surface or bottom. Negative values indicate a loss of bacteria.
Net growth

The net growth rate (r) was determined using the measured bacterial abundance, the residence time and the volumetric flux (see Discussion for details). Net growth rates were small in all regions of the estuary during all months sampled, with values usually falling between 0.005 and -0.005 hr\(^{-1}\) (Fig. 8). The absolute value of r was only higher than 0.01 hr\(^{-1}\) once. This occurred at station 5 in February 1997 when r was equal to –0.02 hr\(^{-1}\). There was no significant seasonal or down-estuary trend in net growth rates over the sampling period. Net growth rates were generally negative at the surface at stations 5 and 3 and generally positive at the surface of stations 4 and 2. Net growth rates in the bottom layer were generally greater than zero at all stations (Fig. 8).

Residence time

Residence time for a conservative component at the surface at station 5 ranged from 2.02 days in February 1997 to 16.83 days at the surface in September 1996 (Table 2). Residence time at station 4 ranged between 0.95 days to 9.75 days while residence time at station 3 ranged from 2.04 days to 10.70 days. Station 2 had the shortest residence time of any region during any sampling period. In October 1996, the bottom water residence time was only 0.62 days. The longest residence time at station 1 was 10.31 days. The time needed to traverse the estuary at the surface from station 5 through station 2 ranged from 8.72 days in February 1997 to 47.12 days in September 1996.
Figure 8. Net growth rate ($r$) for each month at each station. Values are in hr$^{-1}$.

Numbers in upper right corner of each graph indicate station number and surface or bottom.
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Table 3. Monthly dispersion rates and growth rates for each station over entire sampling period. Rates are in hr\(^{-1}\).

<table>
<thead>
<tr>
<th>Month</th>
<th>Depth</th>
<th>Dispersion Station 5</th>
<th>Growth Station 5</th>
<th>Dispersion Station 4</th>
<th>Growth Station 4</th>
<th>Dispersion Station 3</th>
<th>Growth Station 3</th>
<th>Dispersion Station 2</th>
<th>Growth Station 2</th>
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<tr>
<td>June</td>
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<td>Surface</td>
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<td>Surface</td>
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<td>6.40e-03</td>
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<td>Surface</td>
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<td>2.92e-02</td>
<td>4.49e-03</td>
<td>2.98e-02</td>
<td>3.89e-03</td>
<td>2.92e-02</td>
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<td>1.13e-02</td>
<td>1.19e-02</td>
</tr>
<tr>
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<td>Surface</td>
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DISCUSSION

Bacterial fluxes

Horizontal and vertical exchange rates into or out of each reach of the river reflect the sum of all the processes acting on bacterial cells that determine their distribution in the estuary (Fig. 6). The magnitude of the exchange rates depends upon the intensity of the processes acting on the cells. Production and loss rates within a box will go to zero at steady state if circulation is dominant. If biological processes dominate, production rates will be large and positive if growth is greater than removal and large and negative (loss) if removal is greater than growth. Bacterial exchange rates were quite variable in the study, with each region of the estuary sometimes acting as either a sink or a source. The magnitude of the rates was also quite variable. This variability illustrates the complex interactions between circulation and biological processes. Despite this variability, there were some patterns evident. The York River was weakly stratified in seven of the twelve months sampled. During times of stratification, bacterial transport followed water circulation with the transport of cells oriented downstream at the surface and upstream in the bottom layer (Fig. 6). Bacterial exchange rates between the upper and lower layers were generally toward the surface.

The York River was destratified during five of the months sampled. The York was mixed in July 1996, August 1996 and March 1997 due to the periodic oscillation related to the spring tide (Haas 1977). Destratification in November 1996 and May 1997 was unrelated to lunar phase and was probably caused by a wind event or other mixing process. Bacterial exchange rates in the well-mixed estuary were downstream in all but two cases. In July 1996 and in May 1997, there was a transport of cells from station 2
upstream to station 3. Interestingly, station 3 was a large sink for bacteria relative to the other stations during the three sampling periods that the estuary was well mixed over the entire length of the river.

Flux to the mouth

Using this model, we can determine if the York is a source of bacterial cells to the mouth of the river or if it is a sink for cells from outside the mouth. In every month that the York River was stratified, the York delivered cells to the mouth of the river (Fig. 9). When the estuary was well mixed, the net flux of cells into or out of the estuary was variable. The variability illustrates the difference between control of the estuary by tides or by river flow. When the river is stratified, the tide pushes upstream beneath the fresher water flowing downstream and cells are flushed out of the estuary. When the estuary is mixed, the tidal motion involves the entire water column and cells are brought into and out of the estuary depending on the tide. Over the entire year, the average net bacterial flux was out of the estuary to the mouth (Fig. 9). I used the average cell volume of all cells over the entire sampling period to obtain a net total of \( \sim 4 \times 10^4 \) kgC-yr\(^{-1}\). Applying these same factors to Painchaud et al.'s (1996) data I obtained a net export of \( \sim 160 \times 10^4 \) kgC-yr\(^{-1}\) for the St. Lawrence. This much larger export occurs despite the fact that every volume modeled in the St. Lawrence was a sink during Painchaud et al.'s study. The most probable explanation for the difference in magnitude in export of bacterial carbon between the two systems is the much greater freshwater input of the St. Lawrence. The freshwater discharge rate of the St. Lawrence was 12,000 m-s\(^{-1}\) while the freshwater discharge of the York only ranged from \( \sim 12 - 120 \) m-s\(^{-1}\).
Figure 9. Monthly net bacterial carbon export to the mouth of the York River from both surface and bottom volumes of station 2. Values are $10^4$ kg C-year$^{-1}$. 

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Bacterial carbon export

Date

06/96 07/96 08/96 09/96 10/96 11/96 12/96 01/97 02/97 03/97 04/97 05/97

Bacterial carbon export

v = 0.7

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Role of growth and removal

In order to describe the bacterial dynamics and determine the controlling processes, it is necessary to quantify the various biological and physical processes that act upon bacteria within the estuary. The processes relevant to this study are physical dispersion, growth, and removal. I am using the circulation model to quantify dispersion, hydrodynamic residence time, and bacterial fluxes. I also have measurements of bacterial production and biomass from which to obtain specific growth rates. However, I do not have measurements of removal rates. Thus, these rates must be determined from the other information available. The variable \( r \) is the net growth rate, or the difference between removal \((g)\) and growth \((\mu)\) rates. By determining \( r \), the measured growth rates can be used to determine removal rates. Assuming exponential growth,

\[
N_t = N_0 e^{rt}
\]

Which can be rewritten as:

\[
 r = (\ln N_t - \ln N_0)/t.
\]

Where \( N_t \) is observed abundance. \( N_0 \) is the original abundance in that region and \( t \) is residence time. The following equation can be used to find \( N_0 \):

\[
N_o = N_t - (F \times t)
\]

where \( F \) is the net volumetric bacterial flux within the box and \( t \) is the time spent in the box (the residence time). Substituting, we can find a value for \( r \). We then use the fact that \( g = \mu - r \) to find the removal rate for each box. For this study, calculated net growth rates for every station and every month were very low, typically an order of magnitude lower than the growth and removal rates. The apparently tight coupling between growth and removal in the estuary indicates that the removal processes must respond quickly to changes in bacterial abundance (Fig. 10). In this study, the removal term \((g)\) includes
grazing, losses due to viral lysis, sedimentation of cells as well as any other loss terms. Unfortunately, how much of the removal term is accounted for by each process cannot be specified. However, clearance rates can be calculated using the formula

\[ G = N \times g \]

where \( G \) is clearance rate, \( N \) is cell abundance and \( g \) is calculated removal rate. Again, this clearance rate may not be due solely to grazers. However, clearance rates found give a maximum value for clearance by grazers which can be compared to clearance rates of other studies. Clearance rates found in this study ranged from \( 7.0 \times 10^7 \) cells-liter\(^{-1}\)-hour\(^{-1}\) to \( 1.4 \times 10^8 \) cells-liter\(^{-1}\)-hour\(^{-1}\). These values are within the range of those found for other estuarine studies (Coffin and Sharp 1987, Sherr et al. 1989, Coffin and Connolly 1997).

Another possible source of removal was bacterial adsorption onto particles that may then settle out of the water column. To determine the extent to which bacterial removal may be controlled by particle sedimentation, I examined data on total suspended solids (TSS) obtained from the CAST project performed at VIMS during 1996 and 1997 (http://www.vims.edu/physical/castnew/yorkmap.html). CAST TSS data was available for my sampling months from August 1996 to November 1997. There was no significant correlation between TSS and bacterial abundance, production or calculated grazing and clearance rates. This lack of relationship may be influenced by the fact that the CAST data was collected on different days than the data in this study and should be regarded cautiously. However, it does appear likely that most of the removal in the York River during this study was due to grazing.

The mix of removal processes has implications for the fate of carbon assimilated by bacteria. Studies have shown that organic matter consumed by bacteria may not be
Figure 10. Mean growth and grazing rates over the salinity gradient of the York River estuary. Error bars are standard error of the means over the sampling period. Rates are hr$^{-1}$.
transferred to higher trophic levels (Nagata and Kirchman 1991, Miller et al. 1995, Šøndergaard et al. 1995). However, several investigations have shown that microflagellate grazing rates may be as large as bacterial production rates (Coffin and Sharp 1987, Sanders et al. 1989, Sherr et al. 1989). Also, Waterbury and Valois (1993) found that most cells in bacterial communities are resistant to the phages present in that community. Therefore, if the bacterial removal term in the York River is due solely to grazing, then the carbon consumed by bacteria must be transferred to at least the next trophic level.

As stated above, bacterial abundance and production exhibited opposite trends within the York River estuary. Bacterial abundance increased downstream while bacterial production decreased downstream. This implies a strong landward gradient in growth rates. My analysis indicates that this is indeed the case (Fig. 10). Also, the mean annual net growth rate at station 5 is negative and significantly less than the net growth rate at station 2, which is positive (paired t-test, p < 0.1)(Fig. 11). This may explain how biomass may accumulate downstream even though the growth rate is significantly smaller downstream (paired t-test, p < 0.1)(Fig. 10).

Residence time, dispersion rates and biological processes

To compare the importance of biological processes to physical processes in the York River I can compare the growth and removal rates to dispersion. Dispersion is defined as the inverse of the hydrodynamic residence time. The dispersion rate in this study ranged from 0.002 to 0.068 day\(^{-1}\). The net growth rates were lower, ranging from \(~0.0001\) to \(0.05\) day\(^{-1}\) (Table 3). This indicates that in the York River, dispersion controls bacterial distribution within the estuary. However, because rates of growth and
Figure 11. Mean net growth rates (r) at each station over the salinity gradient of the York River estuary. Error bars are standard error of the means over the sampling period. Rates are hr$^{-1}$.
Net growth rate

Surface

Bottom

Station

-0.004 -0.002 0.000 0.002 0.004

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removal are of the same order of magnitude and generally greater than the dispersion rates (ranging from ~ 0.004 to 0.140 day\(^{-1}\)), biological processes are still very important in the York River estuary.

To illustrate the importance of biological processes in the York River estuary, residence time must be considered. The volume of the box and the fluxes of water out of that volume determine the residence time of a constituent in a particular volume of the estuary. Residence time in the York River ranged from 0.62 days in the bottom layer of station 2 in October to almost 17 days in the surface layer of station 5 in September (Table 2). The average residence time over the entire year was between 3 and 8 days in each box. Mean growth rates of about 0.04 hr\(^{-1}\) give a generation time of \(\approx 11\) hours. Therefore, between 6 and 17 generations of bacteria may be produced in each box during the average residence time.

Of course, the small net growth rates indicate that most of the bacteria are removed before significant transit will occur. This quick removal means that those bacterial species with a larger than average net growth rate would be more likely to survive. Thus any change in the estuary such as salinity, temperature or substrate supply that might affect growth rate would affect the community composition. For example, if the growth rate of a particular species was slowed due to exposure to higher salinity water, it might not be able to reproduce quickly enough to maintain its relative biomass within the community. Meanwhile, another species growth rate may not be slowed by exposure to salinity so it may reproduce quickly enough to increase its relative biomass within the community. Thus, the entire bacterial community composition may change significantly during the time it takes to transit the entire length of the estuary.
If a change in community composition did occur, it may help explain the differences in growth rates from the upper estuary to the lower estuary. I have shown that different salinity regimes do appear to have distinct community structure (Ch. 3). Differential growth of favored species is one mechanism leading to these distinct assemblages up- and downriver.

**Comparison to the St. Lawrence**

There were several differences between my study of the York River and Painchaud *et al.*’s study of the St. Lawrence. The St. Lawrence is a larger system than the York, both in river flow and volume. Painchaud *et al.* (1996) modeled the bacterial flux of the St. Lawrence over one time period while I modeled the York over 12 different time periods. Despite these differences, there is still information to be gained by comparing the two systems. The patterns of bacterial fluxes in the York River were quite different from those of the St. Lawrence (Painchaud *et al.* 1996). The St. Lawrence was a sink for bacterial cells in all box volumes, while the different reaches of the York were often sources of cells. In fact, the York was an overall source for bacteria in seven of the twelve months studied. The magnitude of fluxes in the St. Lawrence River was an order of magnitude greater than in the York River. However, the volumetric bacterial flux was on the same order of magnitude for both systems due to the smaller box volumes in the York. Due to the much larger input of freshwater, the residence times for the St. Lawrence were much lower than in the York (~ 140 hours total to ~ 960 hours), despite the much larger box volumes. Also, growth and grazing rates are higher in the York than in the St. Lawrence (0.02 hr\(^{-1}\) – 0.2 hr\(^{-1}\) compared to 0.01 hr\(^{-1}\) to 0.09 hr\(^{-1}\)). Therefore, the ratio of biological processes to dispersion is much greater in the York than in the St.
Lawrence. This means that biological processes in the York River estuary are more significant relative to physical processes than they are in the St. Lawrence estuary.

CONCLUSION

The influence of physical processes on bacterial distribution are difficult to measure directly. Painchaud et al. (1996) improved our understanding of the interactions between circulation and bacterial distribution when they used Officer’s (1980) box model to investigate bacterial dynamics in the St. Lawrence River. My investigation followed Painchaud et al.’s and demonstrated that this model can be successfully used in different estuaries and over seasonal time scales. By using my data with this model, I have determined that removal processes largely control the distribution of bacteria in the York estuary. A small positive net growth rate allows bacterial cells in the lower reaches of the York to accumulate, while a small net negative growth rate keeps the abundance of bacteria upstream low. As Painchaud et al. (1996) pointed out, the net growth rates found using the model are too small to be determined accurately by present experimental techniques. The data needed to run the model are typically collected during most studies, and stream flow data are available for larger rivers through the USGS (http://www.usgs.gov). Therefore, I encourage the use of this model with other systems to allow investigators to gain insights into the interactions between physical dispersion and bacterial abundance.
CHAPTER 3

CHANGES IN BACTERIOPLANKTON METABOLIC CAPABILITIES ALONG A SALINITY GRADIENT IN THE YORK RIVER, VA, ESTUARY
INTRODUCTION

Estuaries are typically regions of steep physical, biological and chemical gradients. Salinity may range from 0 °/oo to near marine salinities over short distances. Depending upon latitude, estuary temperatures may vary considerably on an annual basis. Estuaries are also greatly influenced by human activities. These activities may significantly change the organic matter loadings of an estuary over a short distance (Day et al. 1989). Physical factors such as wind and circulation may change estuarine conditions over a much smaller temporal scale than in the open ocean. These gradients may have a large effect on the composition of the bacterial community as it is carried from the upper reaches of the estuary out to the sea (Ketchum et al. 1952, Painchaud et al. 1996).

Due to these considerations and others, it is difficult to identify the factors that control bacterioplankton abundance and production in an estuary. There have been a number of studies that have attempted to determine what controls bacterial abundance and production in estuaries (e. g. Albright 1983, Coffin and Sharp 1987, Ducklow and Kirchman 1983, Wright and Coffin 1983, 1984, Coffin et al. 1989, Painchaud and Therriault 1989, Findlay et al. 1991, Wright et al. 1987, Ducklow and Shiah 1993, Hoch and Kirchman 1993, Shiah and Ducklow 1994, 1995, Goosen et al. 1997). Some of these
studies focused on a fixed location within the estuary. Others collected data from a transect along the salinity gradient but lumped all the data together to perform data analyses. A few of the studies separated data by position or salinity, but these separations were made without regard to possible differences in microbial community structure. Not surprisingly, strong relationships, such as those seen between bacterial and primary production in the open ocean and in lakes (Fuhrman and Azam 1980, Bird and Kalff 1984, Lancelot and Billen 1984, Bjornsen et al. 1988, Cole et al. 1988, Currie 1990, Simon et al. 1990, White et al. 1991), are not seen in estuarine systems (Albright 1983, Ducklow and Kirchman 1983, Wright and Coffin 1983, 1984, Coffin and Sharp 1987, Painchaud and Therriault 1989, Findlay et al. 1991, Malone et al. 1991, Ducklow and Shiah 1993; but see Goosen et al. 1997). This lack of strong relationships could be due to differences in the metabolic capabilities of different bacterial communities encountered within the estuary in space and time. If one could distinguish between different microbial assemblages, bulk production data could possibly be analyzed in a more insightful and informative manner.

Determining the types of bacteria that exist in any water sample is complicated by several factors. Marine bacteria are difficult to culture (Ferguson et al. 1984). Nucleic acid hybridization, or molecular probing, has been used to detect genes of specific bacterial metabolic potential in marine bacterial consortia without the need for cultivation (Barkay et al. 1989). However, this technique provides no information on whether the activity in question is actually occurring (Walia et al. 1990). Another molecular method that is useful in detecting organisms that are not culturable is polymerase chain reaction (PCR). PCR is used to amplify 16s rRNA sequences and then clone those sequences into
vectors (DeLong et al. 1989, Giovannoni et al. 1990, Ward et al. 1990, Schmidt et al. 1991, Moyer et al. 1994, 1995). Again, these methods give us a great deal of information on the phylogenetic diversity of a sample, but no information on whether the cells are alive, dead, active or inactive.

Lee and Fuhrman (1990, 1991) used purified microbial community DNA to probe for similar sequences in the same and different environments. This gave them a quantitative measure of the relative similarity between populations. However, this method, as well as the other molecular methods discussed above, is very time consuming. The effort and cost required to process samples limits the appeal of these methods. My thesis mainly addresses bacterial abundance, production and determining the factors that control bacterial dynamics. I am less concerned with the species identification of bacteria that are present in the water column. To find the best way to analyze my data, the information I required first was whether the microbial communities I am studying are different from each other.

In the late 1980s, BIOLOG, Inc (Hayward, CA) developed a technique to test bacterial isolates for strain identification using sole carbon source utilization. BIOLOG chose 95 different carbon substrates, based on their ability to distinguish between clinically important species (Bochner 1989). Although the BIOLOG technique was developed for use in clinical microbiology, Garland and Mills (1991) used BIOLOG plates to differentiate between bacterial communities in a marsh creek and in an ocean inlet. Since that time, several other investigators have used BIOLOG plates to characterize environmental communities. Studies have been performed on communities from soil (Garland and Mills 1991, Bossio and Scow 1995, England et al. 1995, Haack et
al. 1995, Vahjen et al. 1995, Wunsche et al. 1995, Knight et al. 1997), plant leaves (Harvey and Miller 1996), tree and crop rhizospheres (Garland and Mills 1991, Garland 1996, Grayston and Campbell 1996), compost (Insam et al. 1996), wastewater (Guckert et al. 1996, Victorio et al. 1996) as well as marine bacterioplankton (Jellet et al. 1996). These studies have generated a great deal of controversy over the use of these plates to provide answers to fundamental questions of microbial diversity (Konopka et al. 1998). However, even critics of BIOLOG plates concede that the plates can be used to differentiate environmental samples that differ from each other in their metabolic capabilities (Konopka et al. 1998).

The purpose of this study was to use BIOLOG plates to distinguish between the metabolic potential of different bacteria communities in an estuary in space and time and to determine the environmental factors that control the separation of communities. I then intended to determine if stratifying my data by community gave me any insights into my data analysis.

MATERIALS AND METHODS

Sampling
York River Samples
Samples were collected bi-monthly from July 1997 to May 1998 at six stations along the salinity gradient of the York River estuary (Fig. 1). Stations were approximately 9 km apart with Station 1 located at the mouth of the York and Station 6 located in freshwater in the Pamunkey River approximately 60 kilometers from the
Figure 1. Map of site locations (1 – 6) and VIMS pier. York River estuary, VA.
mouth. Samples were collected 1 m below the surface at each station using a clean 2.5 liter Niskin bottle. Approximately 750 ml were transferred to a clean 1-liter polycarbonate bottle. Samples were kept in the dark and were brought back to the lab at in situ temperatures. These samples were collected as part of a larger study on bacterioplankton dynamics (see Ch. 1). Water temperature and salinity were measured. Water was also collected for determination of bacterial cell abundance and bacterial activity.

Pier Samples
Samples were collected from the York River weekly from May 1997 to May 1998. Samples were collected 1 m below the surface with a clean 1-liter polycarbonate bottle at the end of the Virginia Institute of Marine Science (VIMS) ferry pier located on the VIMS campus (Fig. 1). BIOLOG plates were immediately inoculated with sample in the lab.

BIOLOG plates
BIOLOG GN plates were used to determine differences in bacterioplankton community metabolic potential. BIOLOG GN microplates (BIOLOG, Inc.) are 96-well microtiter plates containing 95 carbon sources. These sources are supplied in each well in a dried-film form (Bochner 1989). Each well also contains the redox dye tetrazolium violet. As bacteria grow and oxidize each substrate, reduced nicotinamide adenine dinucleotide (NADH) is formed. The tetrazolium violet dye functions as a substitute electron acceptor during this biochemical oxidation (Siedler 1991). The reduced product is a colored, insoluble formazan that is seen as purple color that is quantified
spectrophotometrically. The 96th well is a control that contains tetrazolium dye only. It is assumed that the resultant pattern occurring on the BIOLOG plate is a function of the original community inoculated into the sample wells.

All samples were transferred from the 1-liter sample bottles to the BIOLOG plates using an 8-channel multi-pipette. Care was taken not to touch the interior of the wells to prevent cross-contaminating substrates and tips were discarded between plates to prevent cross-contamination of plates. Due to a problem with condensation forming inside the plate tops, 125 µl of sample were transferred to each well rather than the manufacturer’s recommended 150 µl. Immediately upon inoculation, the zero time-point absorbance of each plate was read before incubating the samples at their *in situ* temperature.

Changes in color development were measured using a single-channel automated spectrophotometric microplate reader (ELx800; BIO-TEK Instruments, Inc.) at a wavelength of 590 nm. Plates were measured approximately every 6 to 24 hours for changes in color development until maximum color development was reached.

Overall color development for each plate was expressed as average well color development (AWCD) after Garland and Mills (1991). \[ AWCD = \Sigma_{i=1}^{95} \frac{(R_i - C)}{95}, \]

where \( R \) is the absorbance of each response well, and \( C \) is the absorbance of the control well. Garland and Mills (1991) suggested using a standard AWCD to eliminate biases caused by differences in initial cell abundance per well. Since color development follows a sigmoidal curve with incubation time, an AWCD value in the middle of the linear phase would give the greatest difference in substrate utilization patterns. Therefore, I used the measured sample when its AWCD was closest to 0.5 ± 0.2 in all cases.
To reduce the influence of rate of color development among the plates, the absorbance value for each well was transformed according to Garland and Mills (1991) by subtracting the absorbance of the control well from the absorbance of each well and dividing by the AWCD. Negative responses were set to zero before analysis.

Hierarchical Cluster Analysis

Hierarchical Cluster Analysis (HCA) was used to determine differences between patterns of substrate utilization with the Ein*Sight v 4.0 software package (InfoMetrix Corp.). The data are assembled into a large matrix. Each sample is placed into one of \( n \) rows with \( p \) columns of variables, in this case the absorbance measurements. The samples are treated as \( n \) points in a \( p \) dimensional space. HCA groups data into clusters having similar attributes by determining the relative distances between pairs of samples in space (Lebart et al. 1984). I used euclidian distance and centroidal clustering in my analysis. When distances are relatively small, the samples are assumed to be similar with respect to the measured variables. Data from HCA are used to construct a dendrogram (Fig. 2). Each of the branches on the far left is called a leaf (e.g. Fig. 2). The shorter the leaf, the greater the similarity and, therefore, the smaller the distance between the samples. Similarity is measured from 0.0 to 1.0 with 1.0 corresponding to an exact duplicate and 0.0 indicating maximum relative dissimilarity, within the data set.

Principal Components Analysis

Principal components analysis (PCA) was used to determine differences between patterns of the substrate utilization profiles (SUP). I used the software package Ein*Sight v 4.0 (InfoMetrix Corp) to perform PCA analysis. To compare BIOLOG plates, we must
Figure 2. Results of hierarchical components analysis on BIOLOG results obtained for samples collected at the VIMS pier. Each leaf is labeled by season, date and temperature. Season is indicated by the first two letters, with S = spring (March, April, May), Su = summer, (June, July, August), F fall (September, October, November) and W = winter, (December, January, February). Numbers at the top indicate percent similarity between paired samples. Samples labeled Su8/26/97-26.7a and b are machine replicates. Samples labeled Su6/10/97-19.8a, b and c are sampling replicates. Samples labeled F10/14/97-22.6a and b are also sampling replicates.
compare samples with 95 variables. PCA is an exploratory tool useful for reducing the number of variables in a given data matrix. PCA makes this possible by collapsing the dimensionality of the data. This is accomplished by projecting the original data onto new axes, or principal components (PCs) (Lebart et al. 1984). These PCs are ranked according to the amount of the variance of the original sample which is accounted for by each PC. PC1 accounts for most of the variance, PC2 accounts for next greatest amount of variance and so on. Basically, the farther apart two samples are in their original (95-dimensional) space, the farther apart they will be in two-dimensional space. This allows us to use PCA analysis to observe relationships between samples by plotting the scores of their PCs in two or more dimensions. Since the first two PCs account for the greatest amount of variance in the samples, they are typically the scores that are plotted.

RESULTS

Patterns of sole carbon source utilization - VIMS pier

Hierarchical cluster analysis

Hierarchical cluster analysis was run on the transformed BIOLOG data collected from the ferry pier to group the data by similarity. The data separated into two main clusters (Fig. 2). Temperature was the most obvious difference between the two clusters. The upper leaves were all samples with water temperature less than 21°C (except for one sample on September 9, 1997) while the lower leaves were mostly (14 of 19) samples from water temperatures greater than 21°C. Similarly, two clusters were also separated by season. Spring and winter samples (March, April, May and December, January,
February respectively) were concentrated in the upper group, while summer and fall samples (June, July, August and September, October, November respectively) were concentrated in the lower group. A spring sample (April 28, 1998) and a winter sample (December 23, 1997) were grouped with the summer and fall groups, and two fall samples (November 25, 1997 and September 9, 1997) and two summer samples (June 3, 1997 and June 10, 1997) were grouped with the spring and winter groups (Fig. 2).

Sample variability was tested in three ways. On August 26, 1997, the same sample was run twice throughout the incubation to test for variability in the spectrophotometer's reading. The similarity between these samples is very close to 1.0 (Fig. 2). On June 10, 1997, three samples were taken from the same 1-liter bottle and incubated together. These samples were grouped by HCA with a similarity > 0.6 (Fig. 2). Finally, two samples were collected in two separate 1-liter bottles on October 14, 1997. These samples were also grouped together at a similarity ≈ 0.6 (Fig. 2). Together, these results suggest that I should concentrate on samples with less than 60% similarity.

**Principal components analysis**

Principal components analysis was run on the 46 transformed BIOLOG data sets from the VIMS pier near the mouth of the York River. PCA was performed with the bimonthly data from May 1997 through May 1998. The samples showed a distinct separation by temperature along the first PC (Fig. 3). PC1 was positive for samples taken when the temperature was > 21°C and negative for samples taken when the temperature was < 21°C. Temperature ranged from 6.7°C to 27.6°C over the course of the sampling period. The sample from April 28, 1998 (water temperature = 16.1°C) was again grouped with the warm water samples. Temperature was positively related to PC1 scores.
Figure 3. Score plot for PC1 and PC2 from PCA of BIOLOG data obtained from samples collected at the VIMS ferry pier. Points are labeled according to temperature.
Figure 4. Linear regression between the first principal component from PCA of the pier data and temperature. \(r^2 = 0.58, p < 0.0001\).
(r² = 0.58; p < 0.0001) (Fig. 4). Temperature was not significantly related to PC2 and the spread along PC2 was about the same for both temperature groups. Two points in the temperature > 21°C group were outside the main cluster along the PC2 axis (September 9, 1997 and July 1, 1997). PC1 accounted for 11.5% of the variance while PC2 accounted for 8.9% of the variance (Table 1). Nearly 60% of the variance was accounted for in the first ten PCs.

Salinity also affected the separation of samples. From May 1997 to May 1998, the salinity at the VIMS pier ranged from 10 °/oo to 23.1 °/oo (VIMS Scientific Data Archive). Salinity was not related to PC1, but was negatively related to the scores of the second PC (r² = 0.33; p < 0.0001). Thus, samples with high salinity values (> 12 °/oo) were relatively lower along the PC2 axis than samples with lower salinity values.

Patterns of sole carbon source utilization - York River transect

Hierarchical cluster analysis

Hierarchical cluster analysis separated the data from the York River transect into five distinct clusters (one of the five is a lone station)(Fig. 5). Examining the five clusters, we see that the top cluster is composed of representatives from all six stations and all six months sampled. However, of the seventeen members of the group, only three of them have salinities above 10 °/oo. These three include two samples from station 5, which over most of the year has a salinity ≈ 5 °/oo. The other sample (station 1 with salinity ≈ 12 °/oo) was also anomalous. Station 1 typically has a salinity > 18 °/oo. The next cluster down is comprised only of station 6 during September 1997. This was a time of low freshwater flow when the salinity at station 6 was > 3 °/oo. The third cluster from
Figure 5. HCA results for BIOLOG data obtained for York River samples. Leaves are labeled by cruise, station number, then salinity. Y13 – July 1997 cruise, Y15 – September 1997 cruise, Y16 – November 1997 cruise, Y17 – January 1998 cruise, Y18 – March 1998 cruise, Y19 – May 1998 cruise (there was no Y14 cruise).
Table 1. The variance accounted for by each of the first twelve principal components of the pier data.

<table>
<thead>
<tr>
<th></th>
<th>% of Variance</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>11.15</td>
<td>11.15</td>
</tr>
<tr>
<td>PC2</td>
<td>8.90</td>
<td>20.05</td>
</tr>
<tr>
<td>PC3</td>
<td>7.99</td>
<td>28.04</td>
</tr>
<tr>
<td>PC4</td>
<td>5.71</td>
<td>33.75</td>
</tr>
<tr>
<td>PC5</td>
<td>5.02</td>
<td>38.77</td>
</tr>
<tr>
<td>PC6</td>
<td>4.70</td>
<td>43.47</td>
</tr>
<tr>
<td>PC7</td>
<td>4.42</td>
<td>47.89</td>
</tr>
<tr>
<td>PC8</td>
<td>4.04</td>
<td>51.93</td>
</tr>
<tr>
<td>PC9</td>
<td>3.45</td>
<td>55.38</td>
</tr>
<tr>
<td>PC10</td>
<td>3.26</td>
<td>58.64</td>
</tr>
<tr>
<td>PC11</td>
<td>3.22</td>
<td>61.86</td>
</tr>
<tr>
<td>PC12</td>
<td>2.82</td>
<td>64.68</td>
</tr>
</tbody>
</table>
the top is composed of stations 1, 2, 3 and 4 during one sampling period (July 1997). The fourth cluster has 4 samples from November and January (with one sample from March) and all but one have salinities greater than 10 °/oo. The last cluster is made up of samples from stations 1, 2, 3, and 4 during September 1997.

The two clusters that are most dissimilar (similarity = 0.0), seem to be split by salinity. The top cluster has fifteen of twenty-two members having a salinity < 10 °/oo. The bottom cluster has fourteen members with only one having salinity < 10 °/oo.

**Principal components analysis**

PCA was run on the bimonthly samples from the six stations along the salinity gradient of the York River (Fig. 6). The first twelve PCs account for more than 70 % of the variance in the data, with PC1 accounting for 16.2% and PC2 accounting for 10.6 % (Table 2). Although there is much overlap, distinct patterns are present in the PCA analysis of the station data (Fig. 6). Stations 1 (mouth) and 6 (freshwater) are separated along both the PC1 and PC2 axes. Station 1 scores are typically negative along the PC1 axis, while station 6 scores are all positive. The one positive station 1 score along the PC1 axis was during May of 1998 after a period of rain when the salinity was very low for that region (= 12.5 °/oo ). There was also separation between stations 1 and 6 along the PC2 axis. Scores for station 1 were generally positive while the scores for station 6 were generally negative. The lone exception occurred in September 1997, characterized by very low rainfall. During most of the year, station 6 is a freshwater station. However, in September 1997, the salinity at station 6 was greater than 3 °/oo.

PCA analysis was unable to clearly differentiate between stations 2, 3 or 4 (Fig. 6). However, there was some interesting information to be gleaned from these data.
Figure 6. PC1 plotted against PC2 for the BIOLOG data collected from the sites along the length of the York River, July 1997 – May 1998. Numbers indicate the station # for each of the six months sampled (Fig. 1).
Table 2. The variance accounted for by each of the first twelve principal components of the York River data.

<table>
<thead>
<tr>
<th>% of Variance</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1 16.20</td>
<td>16.20</td>
</tr>
<tr>
<td>PC2 10.58</td>
<td>26.78</td>
</tr>
<tr>
<td>PC3 7.27</td>
<td>34.05</td>
</tr>
<tr>
<td>PC4 5.98</td>
<td>40.03</td>
</tr>
<tr>
<td>PC5 5.55</td>
<td>45.58</td>
</tr>
<tr>
<td>PC6 4.68</td>
<td>50.25</td>
</tr>
<tr>
<td>PC7 4.19</td>
<td>54.45</td>
</tr>
<tr>
<td>PC8 3.86</td>
<td>58.30</td>
</tr>
<tr>
<td>PC9 3.67</td>
<td>61.97</td>
</tr>
<tr>
<td>PC10 3.51</td>
<td>65.48</td>
</tr>
<tr>
<td>PC11 2.99</td>
<td>68.47</td>
</tr>
<tr>
<td>PC12 2.81</td>
<td>71.28</td>
</tr>
</tbody>
</table>
Most of the scores for all 3 stations were < 1 along PC1. Five of the six station 2 scores were > 1 along the PC2 axis, station 3 had three scores > 1 and three scores below 1 along the PC2 axis, while station 4 had five of its' scores < 1 along the PC2 axis. Station 5 was characterized by being evenly spread about the PC1 axis with three scores less than 0.5 and three scores > 0.5 and by having four of six PC2 scores < -1. These data seem to indicate a general progression from station 1 scores in the upper left quadrant to station 6 scores in the lower right quadrant.

To determine the factors with the greatest influence on pattern development, multiple factor linear regressions were performed between environmental parameters such as temperature, salinity, DOC, DON and chlorophyll a and the scores of the principal components. Temperature and salinity together showed the greatest relationship to both PC1 and PC2. Salinity and temperature accounted for approximately 63% of the variance in PC1 (p < 0.0001)(Fig. 7) and approximately 68% of the variance in PC2 (p < 0.0001)(Fig. 8). Re-examination of the plot of the first two PCs showed a distinct separation of the data points by temperature (Fig. 9). Points from samples with temperatures above 21° C were located above and to the right of those with temperatures below 21° C. Samples were also separated by salinity (Fig. 10). Points from samples with salinity greater than 12°/oo were above and to the left of those with salinity less than 12°/oo.

To relate the utilization of individual carbon substrates to the differences in the sole carbon source utilization patterns, I examined the correlation between the substrate variables and the PCs (Table 3). The higher the correlation, the more important that substrate was to differentiating between samples. A high correlation does not imply that
Figure 7. Three dimensional representation of linear regression between PC1 and temperature and salinity (York River data). \( r^2 = 0.63, p < 0.001 \).
Figure 8. Three dimensional representation of linear regression between PC2 and temperature and salinity (York River data). ($r^2 = 0.68$, $p < 0.001$).
Figure 9. Score plot of PC1 against PC2 of BIOLOG data obtained from water samples collected bi-monthly along the length of the York River, May 1997 – May 1998. Samples are labeled by temperature. Open circles indicate samples collected when the water temperature was greater than 21° C. Closed circles indicate samples collected when the water temperature was less than 21° C. The line was drawn by eye to suggest a general separation of data.
Figure 10. Score plot of PC1 against PC2 of BIOLOG data obtained from water samples collected bi-monthly along the length of the York River, May 1997 – May 1998. Samples are labeled by salinity. Open triangles indicate samples collected with salinity less than 12 °/oo. Closed triangles indicate samples collected with temperature was less than 21°C. The line was drawn by eye to suggest a general separation of data.
Table 3. Correlation coefficient for scores of the first two PCs from the York River data and the transformed measured absorbance for each substrate. All substrates with an $r > 0.5$ are shown ($p < 0.005$). $r$ is the Pearson’s correlation coefficient.

<table>
<thead>
<tr>
<th>PC1 Substrate Name</th>
<th>$r$</th>
<th>PC2 Substrate Name</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D,L-carnitine</td>
<td>0.763</td>
<td>alpha-keto valeric acid</td>
<td>0.663</td>
</tr>
<tr>
<td>D-galacturonic acid</td>
<td>0.720</td>
<td>lactulose</td>
<td>0.654</td>
</tr>
<tr>
<td>β-hydroxybutyric acid</td>
<td>0.718</td>
<td>acetic acid</td>
<td>0.581</td>
</tr>
<tr>
<td>L-pyroglutamic acid</td>
<td>0.692</td>
<td>thymidine</td>
<td>0.565</td>
</tr>
<tr>
<td>2-amino ethanol</td>
<td>0.683</td>
<td>alpha-keto glutamic acid</td>
<td>0.557</td>
</tr>
<tr>
<td>putrescine</td>
<td>0.665</td>
<td>L-proline</td>
<td>0.556</td>
</tr>
<tr>
<td>urocanic acid</td>
<td>0.636</td>
<td>propionic acid</td>
<td>0.529</td>
</tr>
<tr>
<td>m-inositol</td>
<td>0.629</td>
<td>uridine</td>
<td>0.522</td>
</tr>
<tr>
<td>γ-hydroxybutyric acid</td>
<td>0.579</td>
<td>xylitol</td>
<td>0.521</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>0.556</td>
<td>adonitol</td>
<td>0.521</td>
</tr>
<tr>
<td>L-leucine</td>
<td>0.534</td>
<td>L-threonine</td>
<td>0.506</td>
</tr>
<tr>
<td>D-saccharic acid</td>
<td>0.528</td>
<td>Quinic acid</td>
<td>-0.671</td>
</tr>
<tr>
<td>D-psicose</td>
<td>0.518</td>
<td>α-D-glucose</td>
<td>-0.624</td>
</tr>
<tr>
<td>D-melibiose</td>
<td>0.509</td>
<td>sucrose</td>
<td>-0.571</td>
</tr>
<tr>
<td>hydroxy L-proline</td>
<td>0.500</td>
<td>D-saccharic acid</td>
<td>-0.572</td>
</tr>
<tr>
<td>glycogen</td>
<td>-0.697</td>
<td>p-hydroxy phenylacetic acid</td>
<td>-0.559</td>
</tr>
<tr>
<td>glycyll-L-glutamic acid</td>
<td>-0.665</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-cyclodextrin</td>
<td>-0.633</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-gluconic acid</td>
<td>-0.619</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cellobiose</td>
<td>-0.610</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-alanyl-glycine</td>
<td>-0.563</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D, L-lactic acid</td>
<td>-0.557</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-mannitol</td>
<td>-0.515</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-threonine</td>
<td>-0.514</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the carbon source was well utilized. A high correlation simply means that the *difference* in the utilization of that carbon source was highest between samples. Samples with higher PC1 scores showed a higher response to those carbon sources positively correlated to PC1 than samples with lower PC1 scores. For my data, the substrates that were most important in differentiating between sole carbon source utilization were those which explained > 25% of the variance in the data (-0.5 > r > 0.5 using Pearson’s product moment regression). This r is not very large, but no single variable explained more than 43% of the variance in my data.

Examining Fig. 6 and Table 3, we can determine which carbon substrates were most important in differentiating between bacterial communities in the York River. In general, station 1 had the lowest PC1 values. This indicates that the bacterial community at station 1 utilized the substrates that were positively correlated to PC1 relatively *less* than did the bacterial community at station 6. These substrates were comprised of several classes of substrates including carboxylic acids (γ-hydroxybutyric acid, D-galacturonic acid, β-hydroxybutyric acid, and D-saccharic acid), an aromatic acid (urocanic acid), carbohydrates (m-inositol, D-psicose, and D-melibiose), two amines (2-amino ethanol, and putrescine), and amino acids (D,L-carnitine, L-pyroglutamic acid, L-phenylalanine, L-leucine, and hydroxy L-proline). Lower PC1 values at station 1 also indicate that the bacterial community there uses relatively *more* of the substrates that were negatively correlated to PC1 with respect to station 6. These substrates included two carboxylic acids (D-gluconic acid, and D,L-lactic acid), two carbohydrates (cellobiose, and D-mannitol), three amino acids (glycyl-L-glutamic acid, and L-alanyl-glycine) and two polymers (glycogen and α-cyclodextrin).
Station 1 had generally higher PC2 values than did station 6. For the substrates that were positively correlated to PC2, this indicates that the bacterial community at station 1 used relatively more of these substrates than did the community at station 6.

The substrates that correlated with PC2 with an \(-0.5 > r > 0.5\) included several carboxylic acids (\(\alpha\)-ketovaleric acid, acetic acid, \(\alpha\)-ketoglutaric acid, and propionic acid), two aromatic compounds (thymidine and uridine), carbohydrates (lactulose, xylitol, and adonitol), and two amino acids (L-proline, L-threonine). There were also three carboxylic acids (quinic acid, D-saccharic acid, and \(p\)-hydroxyphenylacetic acid) and two carbohydrates (\(\alpha\)-D-glucose and sucrose) that exhibited a negative correlation with PC2. This indicates that the bacterial community at station 1 used relatively less of these substrates than did the community at station 6.

Changes in plate color development over time

Plate color development was measured every twenty-four hours. The change in color development is similar in appearance to a growth curve. To obtain the overall rate of color development, the total color development (\(\Sigma R_i = TCD\)) was exchanged for bacterial cell number in the growth equation: \(\ln(N_t) - \ln(N_0) = kt\) so that we obtain: \(\ln(TCD_t) - \ln(TCD_o) = kt\). This gives a rate of color change analogous to specific growth rate. This equation was applied to all the samples for the York River cruises. Rates for total color development were compared to growth rates obtained from measurements of bacterial cell abundance and incorporation of \(^3\)H-thymidine (Table 4). This comparison was not meant to show that the change in TCD was equal to growth rates, but that the rate of
Table 4. Growth rate and change in total color development (ΔTCD) over the course of the sampling period (July 1997 – May 1998). Growth rates are derived from incorporation of $^3$H-thymidine measurements. Change in total color development is the maximum 24 hour change over the length of the incubation.

<table>
<thead>
<tr>
<th>Station #</th>
<th>July 1997 Growth Rate (day$^{-1}$)</th>
<th>Δ TCD (day$^{-1}$)</th>
<th>September 1997 Growth Rate (day$^{-1}$)</th>
<th>Δ TCD (day$^{-1}$)</th>
<th>November 1997 Growth Rate (day$^{-1}$)</th>
<th>Δ TCD (day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.6</td>
<td>2.1</td>
<td>1.5</td>
<td>0.4</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>1.4</td>
<td>3.5</td>
<td>0.4</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>2.1</td>
<td>4.7</td>
<td>0.5</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>2.8</td>
<td>2.3</td>
<td>4.9</td>
<td>0.6</td>
<td>1.7</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>2.2</td>
<td>3.7</td>
<td>7.1</td>
<td>0.8</td>
<td>1.6</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>5.4</td>
<td>5.0</td>
<td>8.2</td>
<td>0.9</td>
<td>7.0</td>
<td>0.7</td>
</tr>
<tr>
<td>1</td>
<td>0.4</td>
<td>0.1</td>
<td>3.3</td>
<td>0.8</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>0.6</td>
<td>0.2</td>
<td>3.7</td>
<td>0.7</td>
<td>1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>0.1</td>
<td>4.2</td>
<td>0.9</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>4</td>
<td>1.1</td>
<td>0.3</td>
<td>5.7</td>
<td>0.9</td>
<td>4.4</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>0.3</td>
<td>0.4</td>
<td>10.3</td>
<td>1.0</td>
<td>4.6</td>
<td>1.2</td>
</tr>
<tr>
<td>6</td>
<td>1.6</td>
<td>0.6</td>
<td>11.4</td>
<td>1.1</td>
<td>19.9</td>
<td>1.9</td>
</tr>
</tbody>
</table>
color development showed the same pattern as growth rates along the river. Indeed, as seen in Table 4, growth rates typically follow the same pattern over every sampling period. They are relatively low at station 1 and they increase with distance upstream to station 6. This same pattern is seen in the rate of change in TCD. In every case, a linear regression run through the TCD values for a cruise shows a lower TCD at the mouth with TCD increasing with distance upstream. Thus, it appears as though general conclusions on the relative magnitude of growth rates are consistent with the rate of change of color development in BIOLOG plates. However, due to the unrealistic substrate concentrations (Konopka 1998) and other concerns, extreme care should be used when making these types of comparisons.

DISCUSSION

There are several concerns regarding the use of BIOLOG plates with natural samples. Haack et al. (1995) found that cell density in each well must reach $10^8$ ml$^{-1}$ before color development is observed. Most natural environments, including estuaries, have cell densities far below $10^8$ ml$^{-1}$. Thus, BIOLOG plates are basically an enrichment culture and the inoculum will grow a selective subset of the entire population. Also, the substrates included in the BIOLOG plates are not necessarily representative of the substrates available in the natural environment (Konopka et al. 1998). Konopka et al. (1998) clearly summarized these and other concerns regarding the use of BIOLOG with
natural samples. Therefore, attempting to use BIOLOG plates to characterize the
“functional diversity” of microbial communities (Zak et al. 1994) may not be prudent.

BIOLOG plates may still be useful for use with environmental samples, however.
Konopka et al. (1998) concede that BIOLOG plates can distinguish between different
communities in natural samples. Several investigators have successfully used BIOLOG
plates to differentiate between microbial communities (Garland and Mills 1991, Zak et al.
substrate utilization patterns may also be used to identify ecologically relevant bacteria
isolated from environmental samples. McCarthy and Murray (1996) used BIOLOG
plates on 103 Gram-negative isolates cultured from a contaminated aquifer. They were
able to tentatively identify 55 of the isolates and kept records of the carbon source
utilization pattern of the remaining 48 isolates for future characterization. Another
possible use for BIOLOG plates is in substrate addition studies or other studies requiring
long incubations. Bacterial population composition may shift during long incubations
(Suzuki 1998) leading to erroneous results. BIOLOG plates may be quickly and cheaply
used to determine if bacterial communities have changed over the course of incubation.
Finally, by separating different communities, BIOLOG plates can be used to gain insight
into bulk measurements of bacterial properties. Knowing that there are different
microbial assemblages in space and/or time may allow the investigator to stratify data
such that more information may be extracted.

Shiah and Ducklow (1995) found that temperature plays a strong role in
controlling estuarine bacterial dynamics. Therefore, to ensure that bacterial activity is not
Figure 11. Score plot of PC1 against PC2 of BIOLOG data obtained from water samples collected bi-monthly along the length of the York River, May 1997 – May 1998. Open circles represent samples collected when temperature > 21°C and salinity > 12 °/oo; closed circles represent samples collected when temperature > 21°C and salinity < 12 °/oo; open triangles represent samples collected when temperature < 21°C and salinity < 12 °/oo; and closed triangles represent samples collected when temperature < 21°C and salinity > 12 °/oo. Lines were drawn by eye to suggest a general separation of data.
stimulated or retarded relative to their natural environment, I incubated my samples at in situ temperatures. PCA and HCA analysis of the patterns of carbon substrate utilization separated the microbial assemblage of the York River estuary into four communities (Fig. 11). These analyses showed that communities within the York differed by temperature and salinity. Two points should be emphasized here. One is that the four communities may or may not be separated by assemblages of species. The purple color quantified in analysis of BIOLOG plates is due to bacterial oxidation of the substrate. If a species of bacteria is unable to utilize the substrate, due to environmental factors such as extreme temperature or salinity, that species will not reduce the tetrazolium dye and will not add to the measured absorbance of the well. Thus, depending on the environment at the time of collection, a species may be present but not accounted for in the pattern seen on plate. The second point to be made is that, although temperature and salinity separate these communities, these factors may not directly control the bacterial community. Instead, temperature and salinity may foster conditions that indirectly influence the structure of the bacterial community (i.e. primary production).

Stratifying data based on carbon substrate utilization reduces the risk of auto-correlation with the factors that most strongly influence the separation of communities. In my study, salinity and temperature separate the communities. Since many of the environmental parameters may be strongly influenced by salinity and temperature, the risk of auto-correlation is great. Examining the data by community reduces the influence of temperature and salinity and allows us to determine what environmental parameters affect bacterial dynamics within each community. For example, using all the data,
Table 5. Results of correlations (r) between ln abundance and chlorophyll a and between ln abundance and temperature in two communities of the York River.

Community III has temperature < 21° C and salinity > 12‰; Community IV has temperature > 21° C and salinity > 12‰. p < 0.001 in all cases.

<table>
<thead>
<tr>
<th></th>
<th>Temperature</th>
<th>Chlorophyll a</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Abundance (all points)</td>
<td>0.56</td>
<td>NS</td>
</tr>
<tr>
<td>In Abundance (Community III)</td>
<td>0.75</td>
<td>NS</td>
</tr>
<tr>
<td>In Abundance (Community IV)</td>
<td>NS</td>
<td>0.74</td>
</tr>
</tbody>
</table>

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bacterial abundance is not significantly related to Chlorophyll $a$, but is significantly related to temperature (Table 5). If we stratify the data as indicated by the PCA analysis, bacterial abundance is significantly related to temperature only when salinity is greater than $12\%$ and temperature is less than $21^\circ C$. Bacterial abundance and chlorophyll $a$ are significantly and negatively related only where salinity is greater than $12\%$ and temperature is greater than $21^\circ C$ (Table 5). Since abundance and temperature are not significantly related in this community, this cannot be an auto-correlation. This may indicate that bacteria in the higher salinity region of the York may be more dependent upon phytoplankton as their carbon source than the bacteria in low salinity region of the York. This suggestion agrees with my conclusions from Ch. 1.

BIOLOG plates are not capable of determining the species composition of a natural sample. BIOLOG plates cannot identify the types of substrates utilized by a bacterial community in their natural environment (Konopka et al. 1998), but as I have demonstrated here, they are useful in differentiating between microbial communities and in determining the factors that most influence the separation of these communities. Knowing where these separations occur in natural systems may prove to be invaluable in determining how best to stratify measurements of bulk bacterial properties and environmental parameters for analysis. The relative low cost and ease of use makes BIOLOG plates an attractive addition to any study of bacterial dynamics conducted in a natural system over space and/or time.
CHAPTER 4

PROJECT SUMMARY
CONCLUSION

The York River displayed an unusual, and to my knowledge, unique pattern of bacterial properties along the salinity gradient. During every month studied, bacterial abundance in the surface layer displayed a general increase with increasing salinity while bacterial activity in the surface layer tended to decrease with increasing salinity (Ch. 1). This implies a strong landward gradient in growth rates, while at the same time leaving us with the problem of increasing biomass with decreasing activity. A solution to this problem was suggested in Chapter 2. The model showed increased growth rates with distance upstream, but the net growth rate was larger downstream. Thus, bacteria upstream may grow more quickly than bacteria downstream, but they are also removed more quickly than bacteria downstream.

As suggested above, I found that bacterial properties were significantly and positively related to changes in salinity along the salinity gradient. I also found a distinct seasonal cycle in bacterial properties in the York River. Bacterial abundance and production were significantly and positively related to temperature (Ch. 1). Thus, temperature and salinity were the two major controlling factors of bacterial dynamics in the York River estuary during the study. However, this may not be entirely due to direct effects on the bacteria by temperature and salinity. As seen in Chapter 3, there were four distinct communities of bacteria in the York River estuary. Both temperature and salinity differentiated these communities and separated them in time and space. Thus, changes in
bacterial community structure could be the reason that measured bacterial properties changed with temperature and salinity.

On a system-wide basis, there was no significant relationship between bacterial production and phytoplankton biomass or production in the York River (Ch. 1). There may be a temporal lag between bacterial utilization of phytoplankton produced organic material, but this was not evident in the data. Alternatively, bacteria may be dependent upon allochthonous organic material and there may be no relationship with phytoplankton. Another possibility may be that there were different communities of bacteria in the York. This theory was supported by the separation of the York River data set into the four communities suggested by the BIOLOG results. After stratification, a significant relationship was found between bacterial production and chlorophyll $a$ when the temperature was $> 21^\circ C$ and salinity was $> 12\%_0$. My data also showed that bacterial carbon production in the upper estuary was greater than phytoplankton carbon production. Thus, it appears that bacteria in the upper estuary must depend upon allochthonous carbon for their growth substrate. However, bacterial carbon production in the lower estuary was less than phytoplankton production. Unless bacterial growth efficiency was less than $\sim 8\%$, there should be adequate phytoplankton produced carbon in the lower estuary to meet bacterial carbon demand (Ch. 1).

Another of my objectives in this study was to determine the role that physical dispersion plays in the distribution of bacterioplankton within the estuary (Ch. 2). The two-dimensional box model I used gave us some very useful information including bacterial flux, net growth rates and residence times. Bacterial flux within the estuary was quite variable, with the separate regions of the York sometimes acting as a source of
bacterial cells and sometimes acting as a sink for bacterial cells. Net flux of cells was found to be out of the estuary over the course of the entire year.

Mean residence time within the estuary was found to be approximately 21 days in the surface layer and 20 days in the bottom layer. Residence time was needed to compare the importance of physical circulation to biological processes in the distribution of bacterial cells. Dispersion rates, which are the reciprocal of residence time, were found and compared to the rates of biological processes. Dispersion rates were larger than net growth rates. This indicated that physical dispersion dominated the control of bacterial cell distribution dispersion within the estuary (Ch. 2). However, gross rates of growth and removal were greater than dispersion rates. Thus, bacterioplankton in the river have the opportunity to go through several generations during their transit through the estuary. Therefore, there is a strong possibility that shifts in the species assemblage may occur as the bacteria pass through the estuary.

The model was also extremely useful in determining net growth rates. These net rates were very small. At the moment, differences between removal rates and growth rates that are this small are beyond our capacity to measure directly. By calculating net growth rate with the model I was able to determine why increased growth rate in the upper estuary did not lead to increased biomass (see above). Most importantly, the model allowed us to see that removal processes largely control bacterial distribution within the estuary (Ch. 2).

The data in Chapter’s 1 and 2 show the possibility that there may be more than one community of bacteria within the river. I used BIOLOG plates and multi-variate statistics to determine that this was indeed the case (Ch. 3). I was able to separate
bacterial communities over time at the VIMS pier by temperature. In the York, I was able to distinguish four distinct bacterial communities separated by temperature and salinity. This knowledge may provide insights into data analysis, by giving us the ability to examine how different communities of bacteria respond to changes within their environment.

**FUTURE DIRECTIONS**

There are a nearly limitless number of experiments and observations that may be performed within the York River to further our knowledge of bacterial dynamics in this estuary. However, I will suggest only a few here that I believe to be most important. One of the most important things that should be done is to determine bacterial growth efficiency. The trophic status of a system is important in determining whether carbon will be stored in the system or exported from the system. Although there seems to be enough phytoplankton production in the lower York to support the bacterial carbon demand, other investigators have found that the York is supersaturated with CO$_2$ (Raymond and Bauer 1999). This indicates that respiration within the York is greater than production. Both studies took their measurements from the middle of the York in the deep channel. Since large areas of the York are quite shallow, phytoplankton in these areas may be exposed to more light than in the deeper channel. These shallow areas may have a large impact on phytoplankton production, bacterial production and community respiration.
Another important consideration when attempting to determine what controls bacterial dynamics in a system is the percentage of active cells. We determine bacterial abundance by counting all the cells in the sample. However, this gives us no information on whether cells are active or inactive. Active cell abundance may be more closely related to environmental factors than total cell abundance. If we measure the number of active cells as well as the total number of cells, we may be able to see relationships that are currently hidden.

Finally, now that we know there are different communities of bacteria within the estuary, we should use molecular approaches to begin to quantify those differences. Molecular tools may allow us to determine the species composition of these different communities. If we identify and sort active cells using a flow cytometer, we could then use molecular methods to determine the species of bacteria that are active under different conditions. The information obtained from performing the work above would go a long way toward allowing us to understanding the bacterial dynamics of the York River estuary.
APPENDIX I

Bacterial properties may vary over short time periods. During my study, I was concerned that the monthly measurements that I made might not accurately reflect the mean value of the property measured. To investigate the range of variability in bacterial properties over short time scales (hours and days), I decided to collect frequent samples at a fixed location.

From 8:00 AM on July 22, 1997 to 5:00 AM on July 24, 1997, I collected water from the VIMS pier every three hours. Water was collected in a clean 1-liter polycarbonate bottle at the end of the VIMS ferry pier located on the VIMS campus (see Fig. 1, Chapter 3). Samples were immediately brought back to the lab and processed. Temperature and salinity as well as bacterial abundance and activity were measured for each sample. Due to a hard drive failure on my computer unfortunately, bacterial abundance data was lost. Bacterial activity was measured by incorporation of $^3$H-thymidine and $^3$H-leucine (see Methods, Chapter 1 for details).

$^3$H-thymidine incorporation rates ranged from 58.3 to 204.9 pmol-liter$^{-1}$-hr$^{-1}$ (Fig. 1). The mean $^3$H-thymidine incorporation rate was 146.0 pmol-liter$^{-1}$-hr$^{-1}$ with a standard deviation of 40.2 pmol-liter$^{-1}$-hr$^{-1}$. No significant relationship was found with tide, sunlight, salinity or temperature.
Figure 1. Thymidine incorporation rates (pmol-liter$^{-1}$-hour$^{-1}$) from samples collected at the VIMS ferry pier for 48 hour experiment observation experiment. (July 22 – 24). Dashed lines indicate mean ± standard deviation.
Figure 2. Leucine incorporation rates (pmol-liter\(^{-1}\)-hour\(^{-1}\)) from samples collected at the VIMS ferry pier for 48 hour experiment observation experiment. (July 22 – 24).

Dashed lines indicate mean ± standard deviation.
3H-leucine incorporation rates ranged from 340.2 to 1378.2 pmol-liter\(^{-1}\)-hr\(^{-1}\) (Fig. 2). The mean 3H-leucine incorporation rate was 902.7 pmol-liter\(^{-1}\)-hr\(^{-1}\) with a standard deviation of 285.5 pmol-liter\(^{-1}\)-hr\(^{-1}\). Again, no significant relationship was found with tide, sunlight, salinity or temperature.

A series of storms swept through the area during the study. The sky was overcast much of the time, but the wind speed and direction changed several times. Also, there were two periods of rain, one in which it rained lightly but steadily for several hours and another in which it rained very heavily for less than two hours. Thus, the conditions under which this study was conducted were extremely variable. Even so, the coefficient of variation for both 3H-thymidine incorporation and 3H-leucine incorporation was less than 35%.
APPENDIX II

After observing that $^3$H-thymidine incorporation decreased with distance downstream (see Ch. 1), I decided to examine the effect of salinity on bacterial properties to determine if changing salinity directly affected bacterial physiology or growth. Several studies have found that changes in salinity affect bacterial activity. In separate studies, both Mantoura (1987) and Valdés and Albright (1981) found that glucose uptake dropped significantly by the time salinity had reached 5 °/oo. Prieur et al. (1987) determined that halophobic bacteria made up to 50% of the bacterial abundance of freshwater bacterial communities in the Gironde River in France. Painchaud (1995), however, found that increases in salinity up to 10 °/oo did not adversely affect bacterial growth rates. In fact, Painchaud found that bacterial growth rates were stimulated by approximately 50% at 2 °/oo.

To investigate the affect of salinity on freshwater bacteria in the York River, I collected water from a freshwater site of the Pamunkey River near Lester Manor above my station 1 (see Fig. 1, Ch. 1). Water was collected on October 28, 1997 with an acid washed bucket. Approximately 30 l were mixed in a clean (acid washed) 50 l carboy. This water was sub-sampled into 12 clean (acid washed) 2 liter polypropylene bottles (2 liters per sample). As it was being sub-sampled, the water in the carboy was continuously mixed to ensure homogeneity. The samples were then carried back to the
lab in coolers and immediately placed in a large walk-in environmental chamber at the in situ temperature.

The 12 sample bottles were separated into five treatments and one control, with two bottles per treatment or control. Salinity was gradually increased in the treatments by adding a slurry or ~ 5 ml of DI water mixed with 5 g of NaCl. NaCl was added immediately after the 0-time point sampling, after the 6-hour sampling and every 12 hours thereafter until the desired salinity was reached for each treatment. The treatments consisted of two bottles with a final salinity of 2.5 °/oo, two bottles with a final salinity of 5 °/oo, two bottles with a salinity of 10 °/oo, and two bottles with a final salinity of 20 °/oo. I added 80 g of NaCl to the other two treatment bottles immediately after the 0-time point sampling as a “shock” treatment. Approximately 40 ml of water was collected from the samples at the time points indicated in Figs. 1, 2 and 3 for measuring abundance, and bacterial incorporation of ³H-thymidine and ³H-leucine (see Ch. 1, methods for details).

Bacterial abundance at time 0 ranged from approximately 3.6 x 10⁹ cells-liter⁻¹ to 4.3 x 10⁹ cells-liter⁻¹ (Fig. 1). Abundance decreased in the control throughout the experiment. In fact, the abundance in the control and in the 2.5 °/oo treatments generally had the two lowest cell numbers. The abundance in the “shock” treatment fell initially, but recovered and at 192 hours, contained more cells-liter⁻¹ than any other treatment.

There was a general decrease in ³H-thymidine incorporation rates for all treatments other than the “shock” and 10 °/oo treatments (Fig. 2). The “shock” treatment rates fell to less than 5 pmol-liter⁻¹-hour⁻¹ by the 6-hour time point, but began to recover after 48 hours and equaled the control rates by 192 hours. The 10 °/oo treatment rate
increased after 72 hours and remained higher than the rates of the other treatments through the end of the experiment.

The rate of $^{3}$H-leucine incorporation behaved in a similar manner (Fig. 3). The "shock" treatment fell quickly but recovered, while most of the other treatments decreased over the experiment. However, unlike the $^{3}$H-thymidine incorporation rates, the 20 ‰ treatment was also higher than in the other treatments.

In the York River, the average time that is required for a conservative element to traverse the estuary from freshwater to 20 ‰ was more than 20 days (see Ch. 2 for details). This is a much longer amount of time than that seen by the bacteria in the experiment. Even so, there was little apparent effect of the NaCl additions to the bacterial properties. The greater incorporation rates seen at the higher salinities may be a result of a deleterious effect of salinity on bacteriovores. The results of this experiment suggest that changes in salinity do not have an adverse affect on bacterial properties in the York River estuary.
Figure 1. Bacterial abundance (cells-litre$^{-1}$-hour$^{-1}$) over the course of the salinity experiment. Error bars are standard error from replicate samples.
Figure 2. Thymidine incorporation (pmol-liter$^{-1}$-hour$^{-1}$) over the course of the salinity experiment. Error bars are standard error from replicate samples.
Figure 3. Leucine incorporation (pmol-liter\(^{-1}\)-hour\(^{-1}\)) over the course of the salinity experiment. Error bars are standard error from replicate samples.
Griffith et al. (1994) examined attached bacteria in the Chesapeake Bay. They found that attached bacteria made up to 30% of the total abundance. Attached bacteria also accounted for 5–50% of the total thymidine incorporation. In the Columbia River estuary, Crump et al. (1998) found that attached bacteria made up nearly half the total cell abundance and 90% of the total bacterial production. However, the Columbia River estuary has a much stronger flow than the York River estuary with a well-defined turbidity maximum. The residence time of the Columbia is only 1–3 days. Crump et al. (1998) speculate that the hydrodynamics of the Columbia estuary control bacterial dynamics by extending the residence time of particles, and thus attached bacteria, in the turbidity zone, while free-bacteria are flushed out of the estuary too quickly for a distinct population to develop. In the York, however, the residence time is much longer (see Ch. 2). During this study, there was enough time for many (>50) generations of bacteria to be produced within the estuary. Thus, to complete my study of bacterial dynamics in the York River, I needed to determine the relative number of attached bacteria and their contribution to total bacterial production.

To determine the activity and abundance of attached bacteria in the York River, I collected samples at three stations on August 12, 1997. The first station was near the mouth of the York River, the second station was on the Pamunkey River where the
salinity was ~ 1.5 ‰ and the third station was in freshwater in the Pamunkey River. The second station was specifically located to be in or near the turbidity maximum. Samples were collected with an acid-washed 10-liter Niskin bottle. The Niskin was lowered into the water horizontally. SCUBA divers oriented the bottle into the current and tripped the closures. Orienting the Niskin in this fashion was done to ensure that a representative collection of suspended and settling particles was made. The Niskin was then gently emptied into a 13-gallon acid-washed plastic container. The water was covered and sinking particles were allowed to settle for approximately 30 minutes. After settling, supernatant water was removed by siphon from the container being careful not to touch the particulate material on the bottom of the container. As much of the surface water as possible was removed without removing any of the particulate material at the bottom. At this point, about 200 ml of water and particulate material was left. This remaining material, which contained the sinking particles from the entire 10-liter sample as well as the suspended particles in the final 200-ml, was gently shaken for homogeneity and samples were collected for abundance and activity. Activity samples were incubated with thymidine and leucine on board ship and killed after one hour. Abundance samples (40 ml of mixed sample) were killed with 0.2 m filtered 4 % glutaraldehyde (Sigma). Samples were also collected from the whole water supernatant. These samples were then placed on ice before being taken back to the lab for processing.

Abundance was determined by modifying the method of Tso and Taghon (1997) for determining bacterial abundance in muddy sediments. After return to the lab, 5 ml of the abundance sample was added to a 10 ml solution consisting of 5 ml of ice-cold 4 % glutaraldehyde and 5 ml of 0.01 M tetrasodium pyrophosphate-3% NaCl solution (PPI-
NaCl). This mixture was sonicated for 1 minute at 50% cycle, 100-W output power. After sonication, 1 ml of sample was transferred to a vial containing 9 ml of PPI-NaCl solution. Samples were taken from this vial and were filtered and stained using acridine orange (see Methods, Ch. 1 for details). Cell abundance was determined by direct counts of cells. Bacterial activity was measured in the usual way (see Methods, Ch. 1 for details), with special care taken to not remove any of the pellet left after centrifugation. However, rather than 3 sub-samples being assayed, 18 thymidine assays and 24 leucine assays were performed for each station. This was done to minimize the variability due to different amounts of particles being collected in each centrifuge tube.

The abundance of free bacteria was an order of magnitude larger than that of attached bacteria (Fig. 1) at all three stations. Thymidine incorporation of free bacteria was an order of magnitude larger at all three stations as well (Fig. 2). Free bacterial incorporation of leucine was an order of magnitude higher at the first station, two orders of magnitude higher at the second station and nearly three orders of magnitude higher at the third station (Fig. 3).

Free bacteria dominated the number and activity of bacteria during this study. This dominance of free bacteria appeared clear even though I took pains to collect suspended, resuspended and settling particles near the benthic interface. Attached bacteria may play an important role in processes not examined here, but clearly are not a numerically important component of the total bacterial stock or production, even in the particle laden York River estuary.
Figure 1. Abundance of free and attached bacteria collected from the York River in cells-litter$^{-1}$. Station 1 was in freshwater in the Pamunkey River, station 2 in the Pamunkey near West Point at a salinity of ~ 1.5 °/oo, station 1 was at the mouth with a salinity of ~ 20 °/oo.
Figure 2. Thymidine incorporation (pmol-liter$^{-1}$-hour$^{-1}$) of free and attached bacteria collected from the York River. Station 1 was in freshwater in the Pamunkey River, station 2 in the Pamunkey near West Point at a salinity of ~ 1.5 °/oo, station 1 was at the mouth with a salinity of ~ 20 °/oo.
Figure 3. Leucine incorporation (pmol-liter$^{-1}$-hour$^{-1}$) of free and attached bacteria collected from the York River. Station 1 was in freshwater in the Pamunkey River, station 2 in the Pamunkey near West Point at a salinity of $\sim 1.5 \, ^\circ/{\text{o}}$, station 1 was at the mouth with a salinity of $\sim 20 \, ^\circ/{\text{o}}$. 

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